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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 5/00, C07H 21/04	A1	(11) International Publication Number: WO 98/54299 (43) International Publication Date: 3 December 1998 (03.12.98)
(21) International Application Number: PCT/US98/11043 (22) International Filing Date: 28 May 1998 (28.05.98) (30) Priority Data: 60/047,996 28 May 1997 (28.05.97) US (71) Applicant: MASSACHUSETTS INSTITUTE OF TECHNOLOGY (US/US); 77 Massachusetts Avenue, Cambridge, MA 02139 (US). (72) Inventors: HORVITZ, H., Robert; 80 Wendell Street #2, Cambridge, MA 02138 (US). CEOL, Craig; 1st floor, 189 Powderhouse Boulevard, Somerville, MA 02144 (US). LU, Xiaowei; Apartment 23, 12 Pond Lane, Arlington, MA 02174 (US). (74) Agent: BIEKER-BRADY, Kristina; Clark & Elbing LLP, 176 Federal Street, Boston, MA 02110-2214 (US).		(81) Designated States: CA, JP, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.
(54) Title: A TUMOR SUPPRESSOR PATHWAY IN <i>C. ELEGANS</i> (57) Abstract The invention provides novel genes involved in cell fate and cell proliferation, including <i>lin-37</i> , <i>lin-35</i> , <i>lin-53</i> , <i>lin-55</i> , <i>lin-52</i> , and <i>lin-54</i> in multiple species and an E2F-1 gene of <i>C. elegans</i> . Methods for utilizing the genes and encoded proteins are also provided.		

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## A TUMOR SUPPRESSOR PATHWAY IN *C. ELEGANS*

### Background of the Invention

5 The field of the invention is cell proliferation.

Previously we have identified and studied the synthetic multivulva genes in the nematode *elegans*. *C. elegans* is well suited to developmental genetic studies because the entire cell lineage has been mapped and is essentially invariant from one animal to the next. Thus, by comparing the cell  
10 lineage of a wild-type animal to that of a mutant animal, the changes in cellular fates caused by the mutation can be determined.

A number of mutations that alter cell lineage, termed *lin* mutations, were obtained in genetic screens conducted by Horvitz and Sulston in the late 1970's. A subset of the mutations affected the formation of the vulva, a  
15 structure on the ventral surface of *C. elegans* hermaphrodites through which eggs are laid and through which sperm enters during cross-fertilization. Six vulval precursor cells have the potential to undertake a vulval cell lineage as defined by the number and pattern of cell divisions. In a wild type animal only  
20 three of these cells actually undertake vulval cell fates and these three cells generate the 22 cells that make up the adult vulva. In multivulva (*Muv*) animals, most or all of the six vulval precursor cells undertake vulval cell fates. In addition to the cells required for the formation of a normal vulva, these mutant animals generate an excess of cells which cause the formation of raised, vulva-like structures on the ventral surface of the animal. On the other hand, a  
25 vulvaless (*Vul*) phenotype results when no or too few vulval precursor cells adopt vulval cell fates.

Genetic and molecular analyses of *Muv* and *Vul* animals have defined a Ras signal transduction pathway that mediates induction of the

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hermaphrodite vulva. Mutant animals in which this pathway is ectopically activated can display a multivulva phenotype, whereas mutant animals that have reduced Ras pathway signalling can display a vulvaless phenotype. As in the worm, Ras pathways have been found to control cell proliferation in a range of organisms from the yeast *S. cerevisiae* to humans. Members of this pathway, most commonly Ras itself, have been shown to be mutated in a broad range of human cancers.

The synthetic multivulva (synMuv) genes act as negative regulators of worm signalling pathway. The first synthetic multivulva mutant was identified by Horvitz and Sulston. The two genetic loci mutated in this mutant were termed *lin-8* and *lin-9*. Reduction-of-function mutations in both of these loci were required for a multivulva phenotype. Subsequent genetic screens identified a set of loci which fall into the same class as *lin-8*, termed class A genes, and genes which fall into the same class as *lin-9*, termed class B genes. In general, an animal with a reduction-of-function mutation in any class A gene and a reduction-of-function mutation in any class B gene will display a multivulva phenotype yet double mutant animals have wild type vulvae. Thus far four class A loci (*lin-8*, *lin-15A*, *lin-38* and *lin-56*) and ten class B loci (*lin-9*, *lin-15B*, *lin-35*, *lin-36*, *lin-37*, *lin-51*, *lin-52*, *lin-53*, *lin-54* and *lin-55*) have been identified genetically.

Molecular analyses of the synMuv genes have primarily dealt with the class B genes. *lin-15A* and *lin-15B* have been cloned and sequenced. Both genes encode novel protein with no known homologs in other species. *lin-36* and *lin-9* have also been cloned and encode novel proteins.

#### Summary of the Invention

We have cloned synMuv genes in *C. elegans* that are part of a

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pathway which may be used as a genetic and biochemical model system for tumor suppression and, conversely, cancer. Specifically, we have now cloned *lin-35*, *lin-37*, *lin-53*, *lin-52* and *lin-54*. The sequences of *lin-35*, *lin-53*, and *lin-55* all have homology to proteins in the retinoblastoma tumor suppressor pathway family of proteins. This indicates that the synMuv pathway genes and proteins may be used to identify genes which are part of the mammalian pathway (e.g., by finding homologs of novel pathway genes such as *lin-37*, *lin-52*, or *lin-54*) and to identify genes, proteins, and therapeutic compounds which modulate this pathway.

10           The invention also features novel synMuv nucleic acids, proteins, and antibodies which bind these proteins.

          In general, the invention features substantially pure nucleic acid (for example, genomic DNA, cDNA, or synthetic DNA) encoding a mammalian SynMuv polypeptide, as defined below. In related aspects, the invention also features a vector, a cell (e.g., a nematodes mammalian, yeast or bacterial cell), and a transgenic animal or embryo thereof which includes such a substantially pure DNA encoding a SynMuv polypeptide.

          In preferred embodiments, *lin-37*, *lin-35*, *lin-53*, *lin-55*, *lin-52*, *lin-54*, or the *C.elegans* E2F-1 is the gene. In most preferred embodiments the gene is a *C.elegans*/gene or a human or murine *lin-54* gene. In other various preferred embodiments, the cell is a transformed cell. In related aspects, the invention features a animal containing a mutation in a gene which encodes a SynMuv polypeptide that is expressed in or delivered to a cell where a SynMuv gene is know to confer a phenotype.

25           In other aspects, the invention features a DNA sequence substantially identical to a DNA sequence shown in any one of Figs. 3, 5, 7, 8, 10, 12, 14, 26, or 27.

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In another aspect, the invention also features RNA which is encoded by the DNA described herein. Preferably, the RNA is mRNA. In another embodiment the RNA is antisense RNA capable of decreasing at least one biological activity of a SynMuv gene..

5 In another aspect, the invention features a substantially pure polypeptide having a sequence comprising one of the SynMuv amino acid sequences shown in Figs. 2, 4, 6, 8, 9, 11, or 13 or encoded by a nucleic acid of Figs. 26 or 27.

In a second aspect, the invention features a substantially pure DNA  
10 which includes a promoter capable of expressing the SynMuv gene in a cell. In preferred embodiments, the SynMuv gene is *lin-37*, *lin35*, *lin-53*, *lin-55*, *lin-52*, *lin-54*, or *C.elegans* E2F-1.

In preferred embodiments, the promoter is the promoter native to a SynMuv gene. Additionally, transcriptional and translational regulatory  
15 regions are preferably native to a SynMuv gene.

The transgenic cells of the invention are preferably cells which are susceptible to cell death. In preferred embodiments the transgenic cell is a nematode, murine, or human cell.

In another aspect, the invention features a method of modulating cell  
20 death which involves producing a transgenic cell having a transgene encoding a SynMuv polypeptide wherein the transgene is integrated into the genome of the cell and is positioned for expression in the cell wherein the SynMuv transgene is expressed in the cell at a level sufficient to modulating cell death.

In another aspect, the invention features a method of detecting a  
25 SynMuv gene in a cell involving: (a) contacting the SynMuv gene or a portion thereof greater than 9 nucleic acids, preferably greater than 18 nucleic acids in length with a preparation of genomic DNA from the cell under hybridization

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conditions providing detection of DNA sequences having about 50% or greater nucleotide sequence identity to the amino acid encoding DNA sequences of *lin-37*, *lin-35*, *lin-53*, *lin-55*, *lin-52*, *lin-54*, or *C.elegans* E2F-1.

In another aspect, the invention features a method of producing a SynMuv polypeptide which involves: (a) providing a cell transformed with DNA encoding a SynMuv polypeptide positioned for expression in the cell; (b) culturing the cell under conditions for expressing the DNA; and (c) isolating the SynMuv polypeptide. In preferred embodiments the SynMuv polypeptide is expressed by DNA which has a constitutive or inducible promotor. In one embodiment, the promotor is a deterologous promotor.

In another aspect, the invention features substantially pure SynMuv polypeptide. Preferably, the polypeptide includes a greater than 50 amino acid sequence substantially identical to a greater than 50 amino acid sequence shown in any one of Figs. 2, 4, 6, 8, 9, 11, or 13 or encoded by a nucleic acid of Fig. 26 or 27. Most preferably, the polypeptide has at least one biological activity of a SynMuv protein of the immediately foregoing figures.

In another aspect, the invention features a SynMuv gene isolated according to the method involving: (a) providing a sample of DNA; (b) providing a pair of oligonucleotides having sequence homology to a conserved region of a SynMuv gene; (c) combining the pair of oligonucleotides with the cell DNA sample under conditions suitable for polymerase chain reaction-mediated DNA amplification; and (d) isolating the amplified SynMuv gene or fragment thereof.

In preferred embodiments, the amplification is carried out using a reverse-transcription polymerase chain reaction, for example, the RACE method.

In another aspect, the invention features a SynMuv gene isolated



according to the method involving: (a) providing a preparation of DNA; (b) providing a detectably-labelled DNA sequence having homology to a conserved region of a SynMuv gene; (c) contacting the preparation of DNA with the detectably-labelled DNA sequence under hybridization conditions

5 providing detection of genes having 50% or greater nucleotide sequence identity; and (d) identifying a SynMuv gene by its association with the detectable label.

In another aspect, the invention features a SynMuv gene isolated according to the method involving: (a) providing a cell sample; (b) introducing

10 by transformation into the cell sample a candidate SynMuv gene; (c) expressing the candidate SynMuv gene within the cell sample; and (d) determining whether the cell sample exhibits an altered cell proliferative, whereby a response identifies a SynMuv gene.

In another aspect, the invention features a method of identifying a

15 SynMuv gene in a cell, involving: (a) providing a preparation of cellular DNA (for example, from the human genome or a cDNA library (such as a cDNA library isolated from a cell type that is cancerous); (b) providing a detectably-labelled DNA sequence (for example, prepared by the methods of the invention) having homology to a conserved region of a SynMuv gene; (c)

20 contacting the preparation of cellular DNA with the detectably-labelled DNA sequence under hybridization conditions providing detection of genes having 50% nucleotide or greater sequence identity; and (d) identifying a SynMuv gene by its association with the detectable label.

In another aspect, the invention features a method of isolating a

25 SynMuv gene from a recombinant library, involving: (a) providing a recombinant library; (b) contacting the library with a detectably-labelled gene fragment produced according to the PCR method of the invention under

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hybridization conditions providing detection of genes having 50% or greater nucleotide sequence identity; and (c) isolating a SynMuv gene by its association with the detectable label.

In another aspect, the invention features a method of identifying a SynMuv gene involving: (a) providing a cell tissue sample; (b) introducing by transformation into the cell sample a candidate SynMuv gene; (c) expressing the candidate SynMuv gene within the cell sample; and (d) determining whether the cell sample exhibits alteration in cell proliferation, whereby a change in (i.e. modulation of) cell proliferation identifies a SynMuv gene.

Preferably, the cell sample is a cell type which may be assayed for proliferation or a SynMuv phenotype; the candidate SynMuv gene is obtained from a cDNA expression library; and the phenotype involves cell proliferation.

In another aspect, the invention features a method of modulating cell proliferation in an animal wherein the method includes: (a) providing DNA encoding at least one SynMuv polypeptide to a cell; wherein the DNA is integrated into the genome of the cell and is positioned for expression in the cell; and the SynMuv gene is under the control of regulatory sequences suitable for controlled expression of the gene(s); wherein the SynMuv transgene is expressed at a level sufficient to affect cell proliferation relative to a cell lacking the SynMuv transgene. It will be appreciated that SynMuv polypeptides also may be administered directly to modulating cell proliferation.

In a related aspect, the invention features a method of modulating cell proliferation wherein the method involves: (a) producing a cell having integrated in the genome a transgene containing the SynMuv gene under the control of a promoter providing constitutive expression of the SynMuv gene.

In yet another related aspect, the invention features a method of modulating cell death wherein the method involves: (a) producing a cell having

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integrated in the genome a transgene containing the SynMuv gene under the control of a promoter providing controllable expression of the SynMuv gene; and (b) regulating the environment of the cell so that the SynMuv transgene is controllably expressed in the cell. In preferred embodiments, the SynMuv gene is expressed using a tissue-specific or cell type-specific promoter, or by a promoter that is activated by the introduction of an external signal or agent, such as a chemical signal or agent.

In a related aspect, the invention provides a method of modulating cell proliferation in an animal by providing an cell proliferation modulation amount of SynMuv polypeptide.

In another aspect, the invention features a purified antibody which binds specifically to a SynMuv family protein. Such an antibody may be used in any standard immunodetection method for the identification of a SynMuv polypeptide. Preferably, the antibody binds specifically to *lin-37*, *lin-35*, *lin-53*, *lin-55*, *lin-52*, *lin-54*, or *C.elegans* E2F-1. In various embodiments the antibody may react with other SynMuv polypeptides or may be specific for one or a few SynMuv polypeptides. The antibody may be a monoclonal polyclonal antibody.

In another aspect, the invention features a method of identifying a compound which modulates cell proliferation. The method includes (a) providing a cell expressing a polypeptide; and (b) contracting the cell with a candidate compound, and monitoring the expression of a SynMuv gene. An alteration in the level of expression of the SynMuv gene indicates the presence of a compound which modulates cell proliferation. The compound may be an inhibitor or an enhancer of cell proliferation.

By "SynMuv gene" is meant a gene encoding a polypeptide which modulates cell death (inhibiting or enhancing) in a cell or tissue when provided

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by other intracellular or extracellular delivery methods. In preferred embodiments the SynMuv gene is a gene having about 50% or greater nucleotide sequence identity to at least one of the SynMuv amino acid encoding sequences of Figs. 2, 4, 6, 8, 9, 11, or 13 or encoded by the sequence of Fig. 26 or Fig. 27, or portions thereof.

By an "SynMuv gene" is also meant any member of the family of genes characterized by their ability to modulate cell proliferation and having at least 10%, preferably 30%, and most preferably 50% amino acid sequence identity to at least one of the SynMuv protein described herein below.

Representative members of the SynMuv gene family include, the *lin-37*, *lin-35*, *lin-53*, *lin-55*, *lin-52*, *lin-54*, and E2F-1 gene of *C.elegans*, and the *lin-54* genes of the mouse and human.

*lin-54* genes of the invention, in particular, may alternatively be identified as encoding a protein having at least 40% identity to the boxed region in Fig. 13 and encoding at least one of the cystein motifs shown in Section VIII, below.

Specifically excluded from the SynMuv genes of the invention are known retinoblastoma tumor suppressor pathway genes, including the Rb gene, in all species; p107, in human and mouse; and p130 in humans. Also excluded are known E2F genes, including human, murine, and drosophila human E2F genes (e.g., E2F-1, E2F-2, E2F-3, E2F-4, E2F-5, E2F-6).

By "SynMuv protein" is meant a polypeptide encoded by a SynMuv gene.

By "modulating cell proliferation" or "altering cell proliferation" is meant increasing or decreasing the number of cells which undergo cell division in a given cell population or altering the fate of a given cell. It will be appreciated that the degree of modulation provided by a SynMuv or modulating

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compound in a given assay will vary, but that one skilled in the art can determine the statistically significant change in the level of cell proliferation which identifies a SynMuv or a compound which modulates a SynMuv.

By "inhibiting cell proliferation" is meant any decrease in the  
5 number of cells which undergo division relative to an untreated control. Preferably, the decrease is at least 25%, more preferably the decrease is 50%, and most preferably the decrease is at least one-fold.

By "polypeptide" is meant any chain of amino acids, regardless of length or post-translational modification (e.g., glycosylation or  
10 phosphorylation).

By "substantially identical" is meant a polypeptide or nucleic acid exhibiting at least 50%, preferably 85%, more preferably 90%, and most preferably 95% homology to a reference amino acid or nucleic acid sequence. For polypeptides, the length of comparison sequences will generally be at least  
15 16 amino acids, preferably at least 20 amino acids, more preferably at least 25 amino acids, and most preferably 35 amino acids. For nucleic acids, the length of comparison sequences will generally be at least 50 nucleotides, preferably at least 60 nucleotides, more preferably at least 75 nucleotides, and most preferably 110 nucleotides.

20 Sequence identity may be measured using sequence analysis software on the default setting (i.e., Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705). Such software may match similar sequences by assigning degrees of homology to various substitutions,  
25 deletions, and other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine, valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine;

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lysine, arginine; and phenylalanine, tyrosine.

By a "substantially pure polypeptide" is meant a SynMuv polypeptide which has been separated from components which naturally accompany it. Typically, the polypeptide is substantially pure when it is at least 60%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably, the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight, SynMuv polypeptide. A substantially pure SynMuv polypeptide may be obtained, for example, by extraction from a natural source (e.g., a fibroblast, neuronal cell, or lymphocyte cell); by expression of a recombinant nucleic acid encoding a SynMuv polypeptide; or by chemically synthesizing the protein. Purity can be measured by any appropriate method, e.g., those described in column chromatography, polyacrylamide gel electrophoresis, or by HPLC analysis.

A protein is substantially free of naturally associated components when it is separated from those contaminants which accompany it in its natural state. Thus, a protein which is chemically synthesized or produced in a cellular system different from the cell from which it naturally originates will be substantially free from its naturally associated components. Accordingly, substantially pure polypeptides include those derived from eukaryotic organisms but synthesized in *E. coli* or other prokaryotes.

By "substantially pure DNA" is meant DNA that is free of the genes which, in the naturally-occurring genome of the organism from which the DNA of the invention is derived, flank the gene. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote; or which exists as a separate molecule (e.g.,

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a cDNA or a genomic or cDNA fragment produced by PCR or restriction endonuclease digestion) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence.

5 By "transformed cell" is meant a cell into which (or into an ancestor of which) has been introduced, by means of recombinant DNA techniques, a DNA molecule encoding (as used herein) a SynMuv polypeptide.

By "transgene" is meant any piece of DNA which is inserted by artifice into a cell, and becomes part of the genome of the organism which  
10 develops from that cell. Such a transgene may include a gene which is partly or entirely heterologous (i.e., foreign) to the transgenic organism, or may represent a gene homologous to an endogenous gene of the organism. Self replicating units, such as artificial chromosomes, are included.

By "transgenic" is meant any cell which includes a DNA sequence  
15 which is inserted by artifice into a cell and becomes part of the genome of the organism which develops from that cell. As used herein, the transgenic organisms are generally transgenic nematodes or mammals (e.g., *C.elegans* rodents such as rats or mice) and the DNA (transgene) is inserted by artifice into the nuclear genome.

20 By "transformation" is meant any method for introducing foreign molecules into a cell. Microinjection, lipofection, calcium phosphate precipitation, retroviral deliver, electroporation and biolistic transformation are just a few of the teachings which may be used. For example, Biolistic transformation is a method for introducing foreign molecules into a cell using  
25 velocity driven microprojectiles such as tungsten or gold particles. Such velocity-driven methods originate from pressure bursts which include, but are not limited to, helium-driven, air-driven, and gunpowder-driven techniques.

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Biolistic transformation may be applied to the transformation or transfection of a wide variety of cell types and intact tissues including, without limitation, intracellular organelles (e.g., and mitochondria and chloroplasts), bacteria, yeast, fungi, algae, animal tissue, and cultured cells.

5 By "positioned for expression" is meant that the DNA molecule is positioned adjacent to a DNA sequence which directs transcription and translation of the sequence (i.e., facilitates the production of, e.g., a SynMuv polypeptide, a recombinant protein or a RNA molecule).

By "reporter gene" is meant a gene whose expression may be  
10 assayed; such genes include, without limitation,  $\beta$ -glucuronidase (GUS), luciferase, chloramphenicol transacetylase (CAT), and  $\beta$ -galactosidase.

By "promoter" is meant minimal sequence sufficient to direct transcription. Also included in the invention are those promoter elements which are sufficient to render promoter-dependent gene expression controllable  
15 for cell-type specific, tissue-specific or inducible by external signals or agents; such elements may be located in the 5' or 3' regions of the native gene.

By "operably linked" is meant that a gene and a regulatory sequence(s) are connected in such a way as to permit gene expression when the appropriate molecules (e.g., transcriptional activator proteins) are bound to the  
20 regulatory sequence(s).

By "conserved region" is meant any stretch of six or more contiguous amino acids exhibiting at least 30%, preferably 50%, and most preferably 70% amino acid sequence identity between two or more homologs of a SynMuv family member, (e.g., human LIN-54, and nematode LIN-54).

25 By "detectably-labelled" is meant any means for marking and identifying the presence of a molecule, e.g., an oligonucleotide probe or primer, a gene or fragment thereof, or a cDNA molecule. Methods for detectably-



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labelling a molecule are well known in the art and include, without limitation, radioactive labelling (e.g., with an isotope such as  $^{32}\text{P}$  or  $^{35}\text{S}$ ) and nonradioactive labelling (e.g., chemiluminescent labelling, e.g., fluorescein labelling).

5 By "purified antibody" is meant antibody which is at least 60%, by weight, free from proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably, the preparation is at least 75%, more preferably 90%, and most preferably at least 99%, by weight, antibody, e.g., a SynMuv specific antibody. A purified antibody may be obtained, for  
10 example, by affinity chromatography using recombinantly-produced protein or conserved motif peptides and standard techniques.

By "specifically binds" is meant an antibody which recognizes and binds a protein but which does not substantially recognize and bind other molecules in a sample, e.g., a biological sample, which naturally includes  
15 protein.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

#### Brief Description of the Drawings

20 Fig. 1 shows a schematic of a model for synthetic multivulva gene action.

Fig. 2 shows the LIN-37 protein sequence (SEQ ID NO:1).

Fig. 3 shows the *lin-37* cDNA sequence (SEQ ID NO:2).

Fig. 4 shows the LIN-35 protein sequence (SEQ ID NO:3).

25 Fig. 5 shows the *lin-35* cDNA sequence (SEQ ID NO:4).

Fig. 6 shows the LIN-53 protein sequence (SEQ ID NO:5).

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Fig. 7 shows the *lin-53* cDNA sequence (SEQ ID NO:6).

Fig. 8 shows the partial LIN-55 protein sequence (SEQ ID NO:7)  
and the *lin-55* cDNA sequence (SEQ ID NO:8).

Fig. 9 shows the *C. elegans* E2F-1 protein sequence (SEQ ID NO:9).

5 Fig. 10 shows the *C. elegans* E2F-1 cDNA sequence (SEQ ID  
NO:10).

Fig. 11 shows the LIN-52 protein sequence (SEQ ID No:11).

Fig. 12 shows the *lin-52* cDNA sequence (SEQ ID No:12).

Fig. 13 shows the LIN-54 protein sequence (SEQ ID NO:13).

10 Fig. 14 shows the *lin-54* cDNA sequence (SEQ ID NO:14).

Fig. 15 shows a map of the position of *lin-52* on LGIII.

Fig. 16 shows a map of the position of *lin-55* on LGII.

Fig. 17 shows a picture of an RNA blot demonstrating that *lin-37*  
message is present in both embryonic and mixed stage RNAs.

15 Fig. 18 shows a diagram of the *lin-37* gene structure and the  
positions of n758 and n2234.

Fig. 19 shows a diagram of *lin-37::GFP* fusion.

Fig. 20 shows a diagram of constructs used for *lin-37* rescues.

Fig. 21 shows a chart of LIN-37 hydrophobicity.

20 Fig. 22 shows a diagram of a model for *lin-37* function.

Fig. 23 shows a diagram of data obtained using LIN-36, LIN-9, LIN-  
15A, LIN-15B and SNP1 (as a control) in a protein-protein binding assay.

Fig. 24 shows a picture of the strategy for characterization of  
molecular interaction of the SynMuv gene products.

25 Fig. 25 shows a diagram of the strategy for detection of additional  
SynMuv alleles.

Fig. 26 shows the sequence of the *M. musculus* cDNA homolog of

*lin-54* (SEQ ID NO:15).

Fig. 27 shows the sequence of the *H. sapiens* cDNA homolog of *lin-54* (SEQ ID NO:16).

5

## Detailed Description of the Invention

### I. Introduction

We have now cloned *lin-37*, *lin-35*, and *lin-53* and *lin-55*. Data obtained from cloning indicates that the class B genes constitute a tumor suppressor pathway. Accordingly, the synMuv pathway genes, the encoded  
10 proteins, and the worms described herein may be used to identify new tumor suppressors in other species, such as mammals, and may be used to identify therapeutic compounds. Fig. 1 illustrates the placement of our novel genes within the synMuv pathway.

*lin-37* encodes a novel protein and as with the other synMuv genes  
15 cloned and described herein the invention provides the gene, the protein, and mutants derived therefrom (Fig 2, SEQ ID NO 1 and Fig 3, SEQ ID NO 2, respectively.)

*lin-35* has also been cloned. Fig 4 provides the LIN-34 protein sequence (SEQ 3) and Fig 5 provides the *lin-34* sequence (SEQ 4). The  
20 cloning of *lin-35* has shed light on how the class B synMuv genes act to negatively regulate vulval induction. *lin-35* encodes a homolog of the mammalian pocket protein family, which includes retinoblastoma protein (Rb), p107, and p130. This family of proteins has been the subject of intense study since the cloning of Rb in 1986. Rb is a tumor suppressor gene, that is,  
25 mutations that inactivate Rb predispose individuals to tumor formation. Most commonly, inactivation of Rb results in a rare eye cancer, retinoblastoma, although inactivating mutations in Rb have been found in other types of

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tumors. The RB protein is thought to function as a negative regulator of cell cycle progression. A number of molecules which interact, both directly and indirectly, with Rb and the other pocket proteins have been characterized in mammalian cells.

5 Our cloning of *lin-53* (Figs 5 and 7) indicates that *lin-53* encodes a homolog of p48, a protein which has been shown to bind Rb. Although the functional significance of the interaction between p48 and Rb is not fully understood, recent studies suggest p48 may play a role in remodeling chromatin structure.

10 We have also cloned *lin-55* (Fig 8) and found that it encodes a homolog of the DP family of proteins. DP family members, together with E2F proteins, bind DNA at specific sites, thereby regulating the transcription of genes that are essential for cell cycle progression. Pocket proteins such as Rb bind to the DP-E2F complex to repress transcription.

15 We have also cloned *lin-52* (Figs 11 and 12) and *lin-54* (Figs 13 and 14). Like *lin-37*, these genes do not have homology to known tumor suppressor genes. We searched public sequence databases and found human and mouse cDNA clones that are similar to *lin-54*. We have isolated more cDNA clones of the human gene and found that its region of similarity extends  
20 beyond the sequence found in public databases. Due to the sequence similarity, we speculate that *lin-54* shares some common function with the human and mouse genes.

We have also found an E2F-like gene in worms (Figs 9 and 10). This gene, which we are referring to as *C. elegans* E2F-1, was identified  
25 because of its similarity to mammalian E2F genes. We have conducted experiments, which are described below, that suggest that *C. elegans* E2F-1 is a member of the worm synMuv genetic pathway. To summarize, the synMuvs

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act to negatively regulate vulval induction. At a molecular level, vulval induction is controlled by a Ras pathway. Ras pathway members have been shown to mutate in a variety of human cancers to give an increased level of pathway signalling. We have now shown that a pathway related to the tumor suppressor Rb negatively regulates Ras pathway signalling. We have also identified a number of molecules which act as part of this Rb-related pathway. The striking parallel between the Rb pathway in mammals and the Rb-related pathway we have discovered in worms indicates that further characterization of the synthetic multivulva genes and their mammalian homologs, where appropriate, will provide insight into how cell proliferation is regulated in mammals, particularly humans.

Experiments which stem directly from this research include searches for mammalian homologs of the novel synMuv genes. Such homologs may function in activating, enhancing, or otherwise intensifying the effects of tumor suppressors or oncogenes in mammals.

Genetic enhancer or suppressor screens may be performed to identify new genes which may function in/or initiating, enhancing, otherwise interfacing with this Rb-related pathway. In addition, knowing the association provided herein between the synMuv genes and proliferative disease pathways in mammals, one skilled in the art can readily devise drug screens to search for compounds that affect cell proliferation. Specifically, compounds which block the Muv phenotype of synMuv mutant animals are potential antitumor agents. Compounds which stimulate cell division in animals with a single, silent synMuv mutation are likely to be agonists of cell proliferation and may act in a manner analogous to growth factors.

By providing insight regarding the function of the SynMuv genes in

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tumor suppression, Applicants have provided, in concert with generally known molecular biology and nematodes genetic methods, the necessary elements of such methods and the compounds required for the practice of such methods.

## II. Model for synMuv action

- 5           Fig. 1 shows the sites of action of the synMuv genes and the lines indicate sites of negative regulation of the Ras pathway that mediates vulval induction.

## III. Cloning of *lin-52*.

- 10           *lin-52* has been cloned and it encodes a novel predicted protein of amino acids (Figures 11 and 12). The *n771* allele contains a missense mutation in the gene. Reverse-transcriptase-polymerase chain reaction (RT-PCR) analysis of this gene reveals that its RNA is SL2 trans-spliced. SL2 trans-splicing is found most often in RNA from the downstream genes of *C. elegans* operons.

## 15   IV. Cloning of *lin-55*

- lin-55* maps on LGII between *rol-6* and *unc-4*, we first obtained cosmid rescue and identified a candidate gene (Fig. 16). We then proceeded to clone the gene. *lin-55* encodes a protein similar to DP protein family members (Fig 8). A single mutant allele of *lin-55*, *n2994*, has been identified.
- 20   Deficiency studies indicate that the allele results in a partial reduction of LIN-55 function.

## V. Cloning of *lin-37*

          The *lin-37* transcript is approximately 1 kb in size (Fig 3) and is

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present both in embryonic and mixed-staged RNAs as revealed by Northern analysis (Fig 17). A cDNA isolated from the Okkema embryonic cDNA library is about 950 bp in size and can rescue the *lin-37* Muv phenotype when expressed under the control of the *col-10* promoter. (*col-10* encodes a cuticle collagen and is highly expressed in the hypodermis and its precursor cells.)

The predicted polypeptide product of 31.5 kD is novel. We have now determined the sequence of the coding region of the two existing *lin-37* alleles (Fig 18). *n758* contains a splice donor mutation in the first intron and is likely to be a null allele. *n2234* contains a nonsense mutation in the middle of the coding sequence. A *lin-37::GFP* transgene is expressed broadly in embryos and in hypodermal cells and vulval cells throughout larval development, consistent with its cell-nonautonomous site of action (Fig. 19; Hedgecock and Herman, Genetics 141:989-1006, 1995).

Fig. 20 shows the rescue of *lin-37*; Fig. 18 shows the *lin-37* structure; Fig. 21 shows that *lin-37* encodes a novel 32kD hydrophilic protein; and Fig. 22 shows a model for *lin-37* function.

## VI. Cloning of *lin-35* and *lin-53*

We have also rescued *lin-35* and *lin-53* and protein sequences for *lin-35*, *lin-37* and *lin-53*.

*lin-53* is a complex locus containing two transcription units under the control of the same promoter. The 5' transcript is referred to as *lin-53* because it is mutated in *lin-53* mutants, the 3' transcript is about 200 bp apart and encodes a protein 70% identical to the *lin-53* transcription unit and could be functionally related.

The genomic sequence provided does not contain the promoter

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region or the first exon of *lin-53* cDNA. These sequences may, however, be readily obtained using standard cloning techniques.

#### VII. Identification and functional characterization of *C.elegans* E2F-1

We have identified a partial *C. elegans* cDNA clone that shares  
5 similarity with E2F family members. We used this clone to obtain full-length  
cDNA clones of this gene, which we are calling *C.elegans* E2F-1 (Fig 10). We  
have not identified any mutations in *C.elegans* E2F-1 but we have conducted  
experiments that suggest it may function in the worm synMuv pathway.  
Specifically, we have used a technique referred to as "RNA inhibition" to  
10 assess the loss-of-function phenotype of *C.elegans* E2F-1. When injected into  
adult worms, RNA derived from specific gene can inactivate, by an unknown  
mechanism, the same gene in the progeny of the injected worm. We injected  
*C.elegans* E2F-1 RNA into adult worms and found it causes a synthetic Muv  
phenotype in the progeny of injected worms. One may now screen for a  
15 deletion mutation in the *C.elegans* E2F-1 gene using this phenotype to  
determine if it also causes a synthetic Muv phenotype.

#### VIII. Cloning of *lin-54* and identification of human and mouse *lin-54*-like genes

*lin-54* was cloned by standard transformation rescue. It encodes a  
20 predicted protein of 438 amino acids (Figs 13 and 14). The two alleles of this  
gene, *n2231* and *n2990*, are both missense mutations.

In search of public sequence databases, we found human and mouse  
cDNA clones that are derived from genes similar to *lin-54* (Figs 26 and 27).  
We have subsequently isolated more cDNA clones of the human gene in order  
25 to build a complete open reading frame for this gene. Thus far we have an



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open reading frame that is 3.5 kilobases in length. If translated, the human and mouse genes encode proteins that are similar to LIN-54 in a cysteine-rich domain. Within this domain there appear to be two cysteine repeats with the following signatures, where X denotes an amino acid other than cysteine:

5           C X C X<sub>4</sub> C X<sub>4</sub> C X C X<sub>6</sub> C X<sub>2</sub> C X C X<sub>2</sub> C  
             and  
             C X C X<sub>4</sub> C X<sub>4</sub> C X C X<sub>6</sub> C X<sub>3</sub> C X C X<sub>2</sub> C

Although the molecular functions of LIN-54 and these human and mouse proteins are unknown, we believe that the high degree of sequence identity in  
 10 the cysteine-rich domain indicates that the proteins share a common molecular function.

#### IX. Characterization of interactions among the synMuv genes.

Standard yeast 2-hybrid techniques are used to characterize the physical interactions between the synMuv gene products. These 2 hybrid  
 15 systems can also be used to detect therapeutic compounds which disrupt the synMuv protein-protein interactions. We have performed two -hybrid analyses with *lin-9*, *lin-15A*, *lin-15B* and *lin-36* and found that the LIN-36 protein may self-associate but that none of the other proteins appear to interact with each other. The array in

20 Fig. 23 to shows these results.

To further study the protein-protein interactions we have constructed the genetic screen shown in Fig. 24.

This screen should detect synMuv B class mutations which, in combination with a A class mutation, may be lethal to the animal or may cause  
 25 the animal to be sterile. Some synMuv B alleles are sterile without a class A

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mutation in the background. A screen using these mutations would give rise to A<sup>+</sup>; B<sup>-</sup> Muv animals where the B<sup>-</sup> mutation confers sterility.

Using this observation we have also constructed a genetic screen based upon the sterility phenotypes to identify more synMuv genes. This  
5 strategy is shown in Fig. 25.

#### X. SynMuv Protein Expression

SynMuv genes may be expressed in both prokaryotic and eukaryotic cell types. For those SynMuv's which modulate cell proliferation it may be desirable to express the protein under control of an inducible promotor for the  
10 purposes of protein production.

In general, SynMuv proteins according to the invention may be produced by transformation of a suitable host cell with all or part of a SynMuv-encoding cDNA fragment (e.g., the cDNA described above) in a suitable expression vehicle.

15 Those skilled in the field of molecular biology will understand that any of a wide variety of expression systems may be used to provide the recombinant protein. The precise host cell used is not critical to the invention. The SynMuv protein may be produced in a prokaryotic host (e.g., *E. coli*) or in a eukaryotic host (e.g., nematodes, *Saccharomyces cerevisiae*, insect cells, e.g.,  
20 Sf21 cells, or mammalian cells, e.g., COS 1, NIH 3T3, or HeLa cells). Such cells are available from a wide range of sources (e.g., the American Type Culture Collection, Rockland, MD; also, see, e.g., Ausubel et al., Current Protocols in Molecular Biology, John Wiley & Sons, New York, 1994). The  
25 method of transformation or transfection and the choice of expression vehicle will depend on the host system selected. Transformation and transfection methods are described, e.g., in Ausubel et al., (supra); expression vehicles may

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be chosen from those provided, e.g., in Cloning Vectors: A Laboratory Manual (P.H. Pouwels et al., 1985, Supp. 1987).

One preferred expression system is the baculovirus system (using, for example, the vector pBacPAK9) available from Clontech (Palo Alto, CA).

- 5 If desired, this system may be used in conjunction with other protein expression techniques, for example, the myc tag approach described by Evan et al. (Mol. Cell Biol. 5:3610-3616, 1985).

- Alternatively, a SynMuv protein is produced by a stable-transfected mammalian cell line. A number of vectors suitable for stable transfection of  
10 mammalian cells are available to the public, e.g., see Pouwels et al. (supra); methods for constructing such cell lines are also publicly available, e.g., in Ausubel et al. (supra). In one example, cDNA encoding the SynMuv protein is cloned into an expression vector which includes the dihydrofolate reductase (DHFR) gene. Integration of the plasmid and, therefore, the SynMuv protein-  
15 encoding gene into the host cell chromosome is selected for by inclusion of 0.01-300  $\mu$ M methotrexate in the cell culture medium (as described in Ausubel et al., supra). This dominant selection can be accomplished in most cell types. Recombinant protein expression can be increased by DHFR-mediated amplification of the transfected gene. Methods for selecting cell lines bearing  
20 gene amplifications are described in Ausubel et al. (supra); such methods generally involve extended culture in medium containing gradually increasing levels of methotrexate. DHFR-containing expression vectors commonly used for this purpose include pCVSEII-DHFR and pAdD26SV(A) (described in Ausubel et al., supra). Any of the host cells described above or, preferably, a  
25 DHFR-deficient CHO cell line (e.g., CHO DHFR<sup>-</sup> cells, ATCC Accession No. CRL 9096) are among the host cells preferred for DHFR selection of a stable-transfected cell line or DHFR-mediated gene amplification.

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Once the recombinant SynMuv protein is expressed, it is isolated, e.g., using affinity chromatography. In one example, an anti-SynMuv protein antibody (e.g., produced as described herein) may be attached to a column and used to isolate the SynMuv protein. Lysis and fractionation of SynMuv protein-harboring cells prior to affinity chromatography may be performed by standard methods (see, e.g., Ausubel et al., supra).

Once isolated, the recombinant protein can, if desired, be further purified, e.g., by high performance liquid chromatography (see, e.g., Fisher, Laboratory Techniques In Biochemistry And Molecular Biology, eds., Work and Burdon, Elsevier, 1980).

Polypeptides of the invention, particularly short SynMuv protein fragments, can also be produced by chemical synthesis (e.g., by the methods described in Solid Phase Peptide Synthesis, 2nd ed., 1984 The Pierce Chemical Co., Rockford, IL).

These general techniques of polypeptide expression and purification can also be used to produce and isolate useful SynMuv fragments or analogs (described herein).

#### XI. Anti-SynMuv Antibodies

To generate SynMuv-specific antibodies, a SynMuv coding sequence can be expressed as a C-terminal fusion with glutathione S-transferase (GST) (Smith et al., Gene 67:31-40, 1988). The fusion protein can be purified on glutathione-Sepharose beads, eluted with glutathione cleaved with thrombin (at the engineered cleavage site), and purified to the degree necessary for immunization of rabbits. Primary immunizations can be carried out with Freund's complete adjuvant and subsequent immunizations with Freund's incomplete adjuvant. Antibody titres are monitored by Western blot and

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immunoprecipitation analyses using the thrombin-cleaved SynMuv protein fragment of the GST-SynMuv fusion protein. Immune sera are affinity purified using CNBr-Sepharose-coupled SynMuv protein. Antiserum specificity is determined using a panel of unrelated GST proteins (including GSTp53, Rb, HPV-16 E6, and E6-AP) and GST-trypsin (which was generated by PCR using known sequences).

As an alternate or adjunct immunogen to GST fusion proteins, peptides corresponding to relatively unique regions of SynMuv may be generated and coupled to keyhole limpet hemocyanin (KLH) through an introduced C-terminal lysine. Antiserum to each of these peptides is similarly affinity purified on peptides conjugated to BSA, and specificity tested in ELISA and Western blots using peptide conjugates, and by Western blot and immunoprecipitation using SynMuv expressed as a GST fusion protein.

Alternatively, monoclonal antibodies may be prepared using the SynMuv proteins described above and standard hybridoma technology (see, e.g., Kohler et al., Nature 256:495, 1975; Kohler et al., Eur. J. Immunol. 6:511, 1976; Kohler et al., Eur. J. Immunol. 6:292, 1976; Hammerling et al., In Monoclonal Antibodies and T Cell Hybridomas, Elsevier, NY, 1981; Ausubel et al., supra). Once produced, monoclonal antibodies are also tested for specific SynMuv recognition by Western blot or immunoprecipitation analysis (by the methods described in Ausubel et al., supra). Antibodies which specifically recognize SynMuv are considered to be useful in the invention; such antibodies may be used, e.g., in an immunoassay to monitor the level of SynMuv produced by a animal (for example, to determine the amount or subcellular location of SynMuv).

Preferably, antibodies of the invention are produced using fragments of the SynMuv protein which lie outside highly conserved regions and appear

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likely to be antigenic, by criteria such as those provided by the Peptidestructure program of the Genetics Computer Group Sequence Analysis Package (Program Manual for the GCG Package, Version 7, 1991) using the algorithm of Jameson and Wolf (CABIOS 4:181 1988)). In one specific example, such

5 fragments are generated by standard techniques of PCR and cloned into the pGEX expression vector (Ausubel et al., supra). Fusion proteins are expressed in E. coli and purified using a glutathione agarose affinity matrix as described in Ausubel et al. (supra). To attempt to minimize the potential problems of low

10 affinity or specificity of antisera, two or three such fusions are generated for each protein, and each fusion is injected into at least two rabbits. Antisera are raised by injections in a series, preferably including at least three booster injections.

## XII. Identification of Molecules that Modulate SynMuv Protein Expression

Isolation of the SynMuv cDNAs also facilitates the identification of

15 molecules which increase or decrease SynMuv expression. According to one approach, candidate molecules are added at varying concentrations to the culture medium of cells or nematodes expressing SynMuv mRNA. SynMuv expression is then measured, for example, by standard Northern blot analysis (Ausubel et al., supra) using a SynMuv cDNA (or cDNA fragment) as a

20 hybridization probe (see also Table III). The level of SynMuv expression in the presence of the candidate molecule is compared to the level measured for the same cells in the same culture medium but in the absence of the candidate molecule. When nematodes are being used, use of the phenotypes associated with the SynMuv pathway may be used as the primary screen for alteration in

25 protein expression.

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If desired, the effect of candidate modulators on expression may, in the alternative, be measured at the level of SynMuv protein production using the same general approach and standard immunological detection techniques, such as Western blotting or immunoprecipitation with a SynMuv-specific antibody (for example, the SynMuv antibody described herein).

Candidate modulators may be purified (or substantially purified) molecules or may be one component of a mixture of compounds (e.g., an extract or supernatant obtained from cells; Ausubel et al., supra). In a mixed compound assay, SynMuv expression is tested against progressively smaller subsets of the candidate compound pool (e.g., produced by standard purification techniques, e.g., HPLC or FPLC) until a single compound or minimal compound mixture is demonstrated to modulate SynMuv expression.

Alternatively, or in addition, candidate compounds may be screened for those which modulate SynMuv cell death activity. In this approach, the degree of cell proliferation or the SynMuv phenotype in the presence of a candidate compound is compared to the degree of cell death in its absence, under equivalent conditions. Again, such a screen may begin with a pool of candidate compounds, from which one or more useful modulator compounds are isolated in a step-wise fashion. Cell death activity may be measured by any standard assay.

Candidate SynMuv modulators include peptide as well as non-peptide molecules (e.g., peptide or non-peptide molecules found, e.g., in a cell extract, mammalian serum, or growth medium on which mammalian cells have been cultured).

Modulators found to be effective at the level of SynMuv expression or activity may be confirmed as useful in animal models and, if successful, may be used as anti-cancer therapeutics for either the inhibition of cell death.

### XIII. SynMuv Therapy

Because expression levels of SynMuv genes correlates with the levels of cell death, the SynMuv gene also finds use in gene therapy to modulate cell proliferation.

5           Retroviral vectors, adenoviral vectors, adeno-associated viral vectors, or other viral vectors with the appropriate tropism for cells likely to be involved in the cell proliferation disease may be used as a gene transfer delivery system for a therapeutic SynMuv gene construct. Numerous vectors useful for this purpose are generally known (Miller, Human Gene Therapy 15-14, 1990; Friedman, Science 244:1275-1281, 1989; Eglitis and Anderson, 10 BioTechniques 6:608-614, 1988; Tolstoshev and Anderson, Current Opinion in Biotechnology 1:55-61, 1990; Sharp, The Lancet 337:1277-1278, 1991; Cornetta et al., Nucleic Acid Research and Molecular Biology 36:311-322, 1987; Anderson, Science 226:401-409, 1984; Moen, Blood Cells 17:407-416, 15 1991; and Miller and Rosman, Biotechniques 7:980-990, 1989; Le Gal La Salle et al., Science 259:988-990, 1993; and Johnson, Chest 107:77S-83S, 1995). Retroviral vectors are particularly well developed and have been used in clinical settings (Rosenberg et al., N. Engl. J. Med 323:370, 1990; Anderson et al., U.S. Pat. No. 5,399,346).

20           Non-viral approaches may also be employed for the introduction of therapeutic DNA into cells otherwise predicted to undergo insufficient or excess cell proliferation. For example, SynMuv may be introduced into a cell by the techniques of lipofection (Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413, 1987; Ono et al., Neuroscience Lett 117:259, 1990; Brigham et al., 25 Am. J. Med. Sci. 298:278, 1989; Staubinger and Papahadjopoulos, Meth. Enz. 101:512, 1983); asialorosonucoid-polylysine conjugation (Wu and Wu, J. Biol. Chem. 263:14621, 1988; Wu et al., J. Biol. Chem. 264:16985, 1989); or, less



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preferably, microinjection under surgical conditions (Wolff et al., Science 247:1465, 1990).

For any of the above approaches, the therapeutic SynMuv DNA construct is preferably applied to the site of the predicted cell proliferation event (for example, by injection), but may also be applied to tissue in the vicinity of the predicted event or even to a blood vessel supplying the cells predicted to undergo insufficient or excess cell proliferation.

In the gene therapy constructs, SynMuv cDNA expression is directed from any suitable promoter (e.g., the human cytomegalovirus, simian virus 40, or metallothionein promoters), and its production is regulated by any desired regulatory element. For example, if desired, enhancers known to direct preferential gene expression in a particular cell may be used to direct SynMuv expression. Such enhancers include, without limitation, those enhancers which are characterized as tissue or cell specific in their expression.

Alternatively, if a SynMuv genomic clone is utilized as a therapeutic construct (for example, following its isolation by hybridization with the SynMuv cDNA described above), SynMuv expression is regulated by its cognate regulatory sequences or, if desired, by regulatory sequences derived from a heterologous source, e.g., any of the promoters or regulatory elements described above.

Less preferably, SynMuv gene therapy is accomplished by direct administration of the SynMuv mRNA to a cell predicted to undergo excess or insufficient cell proliferation. This mRNA may be produced and isolated by any standard technique, but is most readily produced by in vitro transcription using a SynMuv cDNA under the control of a high efficiency promoter (e.g., the T7 promoter). Administration of SynMuv mRNA to malignant cells is carried out by any of the methods for direct nucleic acid administration

described above.

Ideally, the production of SynMuv protein by any gene therapy approach described above results in a cellular level of SynMuv that is at least equivalent to the normal, cellular level of SynMuv in an unaffected individual.

- 5 Treatment by any SynMuv-mediated gene therapy approach may be combined with more traditional therapies.

Another therapeutic approach included within the invention involves direct administration of recombinant SynMuv protein, either to the site of a predicted or desirable cell proliferation event (for example, by injection) or  
10 systemically by any conventional recombinant protein administration technique. The actual dosage of SynMuv depends on a number of factors, including the size and health of the individual patient, but, generally, between 0.1mg and 100mg inclusive are administered per day to an adult in any pharmaceutically-acceptable formulation.

15 XIV. Administration of SynMuv polypeptides, SynMuv genes, or modulators of SynMuv synthesis or function

A SynMuv protein, gene, or modulator may be administered with a pharmaceutically-acceptable diluent, carrier, or excipient, in unit dosage form. Conventional pharmaceutical practice may be employed to provide suitable  
20 formulations or compositions to administer SynMuv to patients suffering from or presymptomatic for a SynMuv-associated carcinoma. Any appropriate route of administration may be employed, for example, parenteral, intravenous, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, intracisternal, intraperitoneal,  
25 intranasal, aerosol, or oral administration. Therapeutic formulations may be in the form of liquid solutions or suspensions; for oral administration,

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formulations may be in the form of tablets or capsules; and for intranasal formulations, in the form of powders, nasal drops, or aerosols.

Methods well known in the art for making formulations are found in, for example, "Remington's Pharmaceutical Sciences." Formulations for parenteral administration may, for example, contain excipients, sterile water, or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated naphthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the compounds. Other potentially useful parenteral delivery systems for SynMuv modulatory compounds include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation may contain excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or may be oily solutions for administration in the form of nasal drops, or as a gel.

If desired, treatment with a SynMuv protein, gene, or modulatory compound may be combined with more traditional therapies for the disease such as surgery, radiation, or chemotherapy for cancers.

## 20 XV. Detection of A Condition Involving Altered Cell Proliferation

SynMuv polypeptides and nucleic acid sequences find diagnostic use in the detection or monitoring of conditions involving aberrant levels of cell proliferation. A decrease or increase in the level of SynMuv production may provide an indication of a deleterious condition. Levels of SynMuv expression may be assayed by any standard technique. For example, its expression in a biological sample (e.g., a biopsy) may be monitored by standard Northern blot

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analysis or may be aided by PCR (see, e.g., Ausubel et al., supra; PCR Technology: Principles and Applications for DNA Amplification, ed., H.A. Ehrlich, Stockton Press, NY; and Yap and McGee, Nucl. Acids. Res. 19:4294, 1991).

5           Alternatively, a patient sample may be analyzed for one or more mutations in the SynMuv sequences using a mismatch detection approach. Generally, these techniques involve PCR amplification of nucleic acid from the patient sample, followed by identification of the mutation (i.e., mismatch) by either altered hybridization, aberrant electrophoretic gel migration, binding or  
10   cleavage mediated by mismatch binding proteins, or direct nucleic acid sequencing. Any of these techniques may be used to facilitate mutant SynMuv detection, and each is well known in the art; examples of particular techniques are described, without limitation, in Orita et al., Proc. Natl. Acad. Sci. USA 86:2766-2770, (1989); and Sheffield et al., Proc. Natl. Acad. Sci. USA 86:232-  
15   236, (1989).

          In yet another approach, immunoassays are used to detect or monitor SynMuv protein in a biological sample. SynMuv-specific polyclonal or monoclonal antibodies (produced as described above) may be used in any standard immunoassay format (e.g., ELISA, Western blot, or RIA assay) to  
20   measure SynMuv polypeptide levels; again comparison is to wild-type SynMuv levels, and a decrease in SynMuv production is indicative of a condition involving altered cell proliferation. Examples of immunoassays are described, e.g., in Ausubel et al., supra. Immunohistochemical techniques may also be utilized for SynMuv detection. For example, a tissue sample may be obtained  
25   from a patient, and a section stained for the presence of SynMuv using an anti-SynMuv antibody and any standard detection system (e.g., one which includes a secondary antibody conjugated to horseradish peroxidase). General guidance

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regarding such techniques can be found in, e.g., Bancroft and Stevens (Theory and Practice of Histological Techniques, Churchill Livingstone, 1982) and Ausubel et al. (supra).

In one preferred example, a combined diagnostic method may be employed that begins with an evaluation of SynMuv protein production (for example, by immunological techniques or the protein truncation test (Hogerrorst, F.B.L., et al., Nature Genetics 10:208-212 (1995) and also includes a nucleic acid-based detection technique designed to identify more subtle SynMuv mutations (for example, point mutations). As described above, a number of mismatch detection assays are available to those skilled in the art, and any preferred technique may be used (see above). By this approach, mutations in SynMuv may be detected that either result in loss of SynMuv expression or loss of SynMuv biological activity.

Mismatch detection assays also provide the opportunity to diagnose a SynMuv-mediated predisposition to diseases of cell proliferation. For example, a patient heterozygous for a SynMuv mutation may show no clinical symptoms and yet possess a higher than normal probability of developing one or more types of diseases. Given this diagnosis, a patient may take precautions to minimize their exposure to adverse environmental factors (for example, UV exposure or chemical mutagens) and to carefully monitor their medical condition (for example, through frequent physical examinations). This type of SynMuv diagnostic approach may also be used to detect SynMuv mutations in prenatal screens.

The SynMuv diagnostic assays described above may be carried out using any biological sample (for example, any biopsy sample or bodily fluid or tissue) in which SynMuv is normally expressed. Identification of a mutant SynMuv gene may also be assayed using these sources for test samples.

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Alternatively, a SynMuv mutation, particularly as part of a diagnosis for predisposition to SynMuv-associated proliferative disease, may be tested using a DNA sample from any cell, for example, by mismatch detection techniques; preferably, the DNA sample is subjected to PCR amplification prior to analysis.

5

### Other Embodiments

In other embodiments, the invention includes any protein which is substantially identical to a mammalia SynMuv polypeptides (Figs. 2, 4, 6, 8, 9, 11, 13, or encoded by a nucleic acid of Figs 26 or 27; such homologs include other substantially pure naturally-occurring mammalian SynMuv proteins as well as allelic variants; natural mutants; induced mutants; DNA sequences which encode proteins and also hybridize to the SynMuv DNA sequences of (Figs. 3, 5, 7, 8, 10, 12, 14, 26, or 27, under high stringency conditions or, less preferably, under low stringency conditions (e.g., washing at 2X SSC at 40°C with a probe length of at least 40 nucleotides); and proteins specifically bound by antisera directed to a SynMuv polypeptide. The term also includes chimeric polypeptides that include a SynMuv portion.

The invention further includes analogs of any naturally-occurring SynMuv polypeptide. Analogs can differ from the naturally-occurring SynMuv protein by amino acid sequence differences, by post-translational modifications, or by both. Analogs of the invention will generally exhibit at least 85%, more preferably 90%, and most preferably 95% or even 99% identity with all or part of a naturally-occurring SynMuv amino acid sequence. The length of sequence comparison is at least 15 amino acid residues, preferably at least 25 amino acid residues, and more preferably more than 35 amino acid residues. Modifications include in vivo and in vitro chemical derivatization of polypeptides, e.g., acetylation, carboxylation,

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phosphorylation, or glycosylation; such modifications may occur during polypeptide synthesis or processing or following treatment with isolated modifying enzymes. Analogs can also differ from the naturally-occurring SynMuv polypeptide by alterations in primary sequence. These include genetic variants, both natural and induced (for example, resulting from random mutagenesis by irradiation or exposure to ethanemethylsulfate or by site-specific mutagenesis as described in Sambrook, Fritsch and Maniatis, Molecular Cloning: A Laboratory Manual (2d ed.), CSH Press, 1989, or Ausubel et al., supra). Also included are cyclized peptides, molecules, and analogs which contain residues other than L-amino acids, e.g., D-amino acids or non-naturally occurring or synthetic amino acids, e.g.,  $\beta$  or  $\gamma$  amino acids.

In addition to full-length polypeptides, the invention also includes SynMuv polypeptide fragments. As used herein, the term "fragment," means at least 20 contiguous amino acids, preferably at least 30 contiguous amino acids, more preferably at least 50 contiguous amino acids, and most preferably at least 60 to 80 or more contiguous amino acids. Fragments of SynMuv polypeptides can be generated by methods known to those skilled in the art or may result from normal protein processing (e.g., removal of amino acids from the nascent polypeptide that are not required for biological activity or removal of amino acids by alternative mRNA splicing or alternative protein processing events).

Preferable fragments or analogs according to the invention are those which facilitate specific detection of a SynMuv nucleic acid or amino acid sequence in a sample to be diagnosed.

All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each independent publication or patent application was specifically and individually indicated to be incorporated by reference.

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What is claimed is:



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Claims

1. A substantially pure nucleic acid encoding a SynMuv polypeptide selected from the group consisting of LIN-37, LIN-35, LIN-55, LIN-53, LIN-52, LIN-54, and E2F-1.
- 5           2. The nucleic acid of claim 1, wherein said nucleic acid comprises nucleic acid encoding LIN-54.
3. The nucleic acid of claim 1, wherein said nucleic acid encodes LIN-37, LIN-52 or LIN-54.
4. The nucleic acid of claim 1, wherein said nucleic acid is cDNA.
- 10           5. The nucleic acid of claim 1, wherein said nucleic acid is *C.elegans* DNA.
6. The nucleic acid of claim 1, wherein said nucleic acid is human DNA.
7. A substantially pure DNA encoding an amino acid sequence selected from the group consisting of SEQ ID NOS:1, 3, 5, 7, 9, 11, and 13.
- 15           8. A substantially pure DNA having the sequence of SEQ ID NO:15, or degenerate variants thereof.
9. A substantially pure DNA having the sequence of SEQ ID NO:16,

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or degenerate variants thereof.

10. A substantially pure nucleic acid comprising nucleic acid having about 50% or greater nucleotide sequence identity to the DNA sequence of selected from the group consisting of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 15,  
5 and 16.

11. The nucleic acid of claim 1, wherein said DNA is operably linked to regulatory sequences for expression of said polypeptide and wherein said regulatory sequences comprise a promoter.

12. The nucleic acid of claim 11, wherein said promoter is a  
10 constitutive promoter.

13. The nucleic acid of claim 11, wherein said promoter is inducible by one or more external agents.

14. The nucleic acid of claim 11, wherein said promoter is cell-type specific.

15. A vector comprising the nucleic acid of claim 1, said vector being capable of directing expression of the peptide encoded by said DNA in a vector-containing cell.

16. A cell which contains the nucleic acid encoding a SynMuv polypeptide selected from the group consisting of LIN-37, LIN-35, LIN-55,  
20 LIN-53, LIN-52, LIN-54, and E2F-1.

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17. The cell of claim 16, said cell being present in a patient having a cell proliferation disease.

18. A transgenic cell which contains the nucleic acid encoding a SynMuv polypeptide selected from the group consisting of LIN-37, LIN-35,  
5 LIN-55, LIN-53, LIN-52, LIN-54, and E2F-1.

19. A substantially pure mammalian SynMuv polypeptide.

20. The polypeptide of claim 19, wherein said polypeptide is LIN-54 polypeptide.

21. A therapeutic composition comprising as an active ingredient a  
10 SynMuv polypeptide, said SynMuv polypeptide being formulated in a physiologically acceptable carrier.

22. A method of modulating cell proliferation of a cell, said method comprising administering to said cell a proliferation modulating amount of SynMuv polypeptide.

15 23. The method of claim 22, wherein said cell is in a mammal.

24. The method of claim 23, wherein said mammal is a human.

25. A SynMuv gene isolated according to the method comprising:  
(a) providing a cell sample;  
(b) introducing by transformation into said cell sample a candidate

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SynMuv gene;

(c) expressing said candidate SynMuv gene within said cell sample;

and

(d) determining whether said cell sample exhibits an altered cell

5 proliferation response, whereby an altered level of cell proliferation identifies a SynMuv gene.

26. A purified antibody which binds specifically to a SynMuv family protein.

27. A method of identifying a compound which modulates cell  
10 proliferation, said method comprising (a) providing a cell expressing a SynMuv polypeptide; and (b) contracting said cell with a candidate compound and monitoring the expression of a SynMuv gene, an alteration in the level of expression of said gene indicating the presence of a compound which modulates cell proliferation.

1:5 28. A method of diagnosing an animal for the presence of an cell proliferation disease or an increased likelihood of developing a cell proliferation disease, said method comprising isolating a sample of nucleic acid from said animal and determining whether said nucleic acid comprises a mutated SynMuv gene, a mutation in said nucleic acid being an indication that  
20 said animal has an cell proliferation disease or an increased likelihood of developing a cell proliferation disease.

29. A method of diagnosing an animal for the presence of a cell proliferation disease or an increased likelihood of developing a cell

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proliferation disease, said method comprising measuring SynMuv gene expression in a sample from said animal, an alteration in said expression relative to a sample from an unaffected animal being an indication that said animal has a cell proliferation disease or increased likelihood of developing a cell proliferation disease.

30. The method of claim 28 or 29, wherein said SynMuv gene is *lin-54*.

31. The method of claim 28 or 29, wherein said gene expression is measured by assaying the amount of SynMuv polypeptide in said sample.

10 32. The method of claim 31, wherein said SynMuv polypeptide is measured by immunological methods.

33. The method of claim 29, wherein said SynMuv gene expression is measured by assaying the amount of SynMuv RNA in said sample.

# A Model for Synthetic Multivulva Gene Action

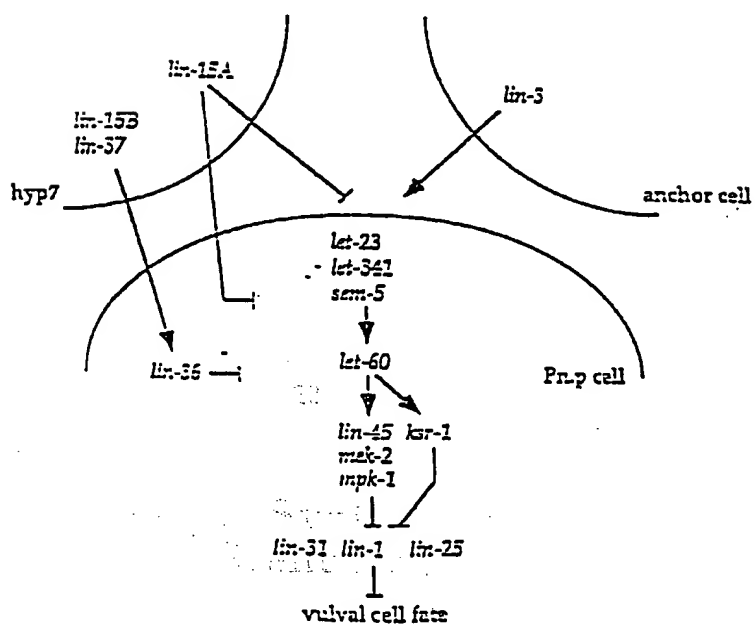


FIG. 1

>lin-37 protein sequence  
MSEIDPLAEFLPEDGDRNARQNDPLISGGPLPLESPSRKLTSLLSYDFTVPESPDIM  
FARKRLGNLLTHKHPSEEGVLPEDYTRADEEPGRQGRPFGRPRKMPREHSTSLM  
ESPRATMTRDSKMFELRGHFEMACGRFEENSLGRAWVKGHMNNETEPKAQRTD  
YAPNLAVDYLACREHRMFRPDNSIFELPPPSRDEFDATVDPRYETDLKNEYRH  
WKQVKNKGWCAHQRRRTAPHARSIALNKYQPGESKTVEQALGLI

SEQ ID NO:1

FIG. 2

>lin-37 cDNA sequence  
ATGTCAGAAATAGATCCACTTGCCGAGTTCTTGCTTCCAGAAGACCGAGATCGAA  
ATGCTCGTCAAAATGATCCATTGATAGCGGAGGTCCACTTCCATTGGAATCGCCA  
AGCAGAAAACTCACATCCCTGTTATCCTATGATCCGACAGTTCGGGAGTCACCGGA  
TATGAAATTCGCCAGAAAACGTCTGGGAAATCTGCTGACAACCATAAAAACATCA  
CCCATCGGAAATAATTGGAGTACTCCCAGAAAGATTATACTCGTGCTGATGAAGAG  
CCCCGGCGCCAAGGACGTCCACCAGGTCCCTCGTAAGATGCCCGCTACGAATCTT  
CAACTTCACTTATGGAATCACCAAGCAAGACTATGACTCGTGATTCTAAAATTAT  
GTTTGAATTGCGTGGAAAACCATTCGAAATGATAGCTGGACGTTTTGAAGAAGA  
ATATTCACTTGGTAGAGCATGGGTAAAGGACACATGAATAATGAATATGAACC  
AATAAAAGCTCAAGGACAGACTATGCACCGAATCTGGCTGTTGATTATCTTGCA  
TGTCGCGAGATTATCGAATGCCACGTCCAGATAAATCAATTCCTGAGCTGCCAAT  
TGTTCCATCTAGAATCGATGAATTCGACGCTACAGTCGATCCAAGATATGAAACA  
GATTTGAAAAATGAATACATTTCGTCAATTGGAAACAAGTCAAAAAAGGTTGGTG  
TGCTCATCAACGTCGTCCGACTGCTCCCCATGCAAGAAGCATAGCATTAAATCAACA  
AAATCTACCAGCTGGAGAGTCGAAAACGTGTCGAGCAAGCACTTGGTCTTATTTA  
AATATTCTAACATGTAATTTCAATTTATCTCTTACTTTCTGATCTTGCTATCACA  
TGCTCTTATTTCAAAAATCTCACTTTAAAATTCATATAAATAATGGGTTTATT  
CAAATACATCATCTTGAC

SEQ ID NO:2

FIG. 3

>LIN-35 protein sequence  
MPKRAADEPGTSTTDFTEQSPFDVLAGTETTDICEEPPAKRIDLDKQEFNGGVQ  
SCGLENESELTQMTKQETECNNEARREEDEEQDEDSRTSMPPALGEDDDYEDDA  
DSFDKNTNTPFPSSQSFLECCRAANLPNDVTCAWETYNHAVQRVSLGSES AWQLS  
AIYYLLSKGKRRGKTRKIQPPVSLTIANSEFISVAEMILDKTARFVEIHSRKIR  
RYQEYRRRQCEGLAVSCVFKNFCRFCKFEHKVGSENCPSSEHFTVLWTSFLVMK  
SRMTVDDLISNYQLLFSLDQNTTEMCSMKEGVHHLNQKFVEDLLENDCTIRALCT  
QFGGSVLDARHFSDFHAKMENTGEPSTWNTQEFRDLNVPKTAIENYLLQSGSD  
ERLTPSVEDFSKIFSPDTYSADILKVSYSGRFRDAEFLTKISNNHCLEKALGGK  
VASEKLVTSKEQPRVPCVEYNLELGNYPDDLESNNQSLYNRLTKESWKLNSKLE  
EVCGTMSDSPMATILLKSDENITNKFERTLSAELGETENENTPKYHYNVKLELVLI  
FMKIVAEIKKKYREEDLLNTRREEFLDSVFCFCVELILVSNGYDRPFPWSAELCG  
VHPFMFHKVIDLMTHEKQLSRQIVQHFSRIETVIEYFSWKS DSPLPWPMVVRCPF  
AHFQEFGEDWADKLNYSFKEFTPKKPDLLRDELGRPIVQNPQTSRTLRIFLKRTYFT  
AARRLQDLTDRVSMGARAKSQCSLFDYLLRNDTLIFMDRHLDDQLLCCVFVMIKI  
NESSMLFTEIMAQYRRQSANSLLVYRSVTVFQEQLNPENPQAVNTKETILERLEGPO  
KEKTTVDIKYYNTEFRDRKYEGQDSASDEDLMENPVATESGLMPVRVYLTHKLS  
IQTLPKTKHGKSKQERALANLEKSGITAMERSGD SEQ ID NO:3

FIG. 4

FIG. 5



>lin-35 cDNA sequence  
CCAAAGTTTGAGACTGGTAAAACTTCTTCAATATGCCGAAACGAGCAGCCGATGA  
CCCTGGAACATCAACAACTGACCCATTTCACGAGCAAGGCCATTGCGCGGTGT  
TAGCCGGCCACCGAGACACCGGATACAATATGTGAAGAGCCACCACCAAAACGAA  
TCGACTTAGATATAAAGCAAAGAAATTCATGGTGGAGTCCAAAGTGGAGCCCTGA  
TTAAAAATGAATCCGAATTGACTCAAAATGACAATCAAAACAAGAAACAGAAAGG  
AAACATAAATGAAGCTAGACGAGAAGAAAGAAGACGAAAGAAACAGAAAGG  
TTCCAGAACATCAATGCCACCTGCCATTGGGAGAAGATGATGATTATGAGGAGGAT  
GATGCTGATAGTTTTATTGATAAACTAATACACCGCCACCATCACAATCATTTT  
TGGAAGGATGTGAGCAGCTAATTTACC.AAATGACATTGTTACTGCTGCATGGGA  
AACGTACAACCACCGCGTTCAACGCGTTTCTCTTGAGGGTTCGGAATCGCGGTGCCA  
ACTATCAGCAATTTACTATTATCTTCTATCAAAAGGAATAAAACGTGCTGGAAA  
AACAATCCGTATTTCTATTCAACCGTTTCTGTTTCGATACTTACAATTGCCAACT  
CATTGACATATCCGTTGCTGAAATGCTTGACAAAACTGCTCGATTGTGGAAAT  
TATACATTCCAGAAAAATTCGTGTTATCAAGAAATATATTGACGAATTCGAAGA  
AGGACTCGCAGTTTCTGTGTGATATCAAAAAAGTTTTGCCGAATTTTCTGCAAA  
ATATTGAGGAGATCAAAAGTTGGATCCGAAAAATTGTCCATCTTCTCATGAACCTT  
TTACGGTTCTTTGGACATCTTTCTGGTGATGAAAAAGTCGAATGACAGTGGACGAT  
TTGATTTCAAATTATCAACTTCTTTTCAATACTTGATCAAGTATATACCGAA  
ATGTGTTCAATGAAAGAGGGAATAGTTCATCATTTGAATCAAAAATTTGTTGA  
AGATCTTCTTGAAAATGATTGTACGATTATTGAGCTCTTTGCACACAATTTGGT  
GGAAGTGTCTTGATGCACGGCACTTTCTGATCATACTTTAAGAAAAATGGAGA  
AGACTGGAATTCGTCCACTTGGAAATTTCAAGAGTTTCGAGATTGATCATGAA  
CGTTCCAAAAACGGCATATGAGAATTATCTATTGCAACGTGGAAGTATTGATGA  
GCGGATTTTCAATCCAAAGCGTTGAGGACTTTTCAAAAAATTTCCAATCCCCGGACA  
CATACTCAGTAGCAGATATTTCTCAAAGTGTCTTACTCTGGAAGACGTTTCCGTGAT  
GCAGAATTTCTTACAAAAATCTCAAATAATCATTGTCTGGAAAAGTTGGCATTAA  
GGTGGAAAAGTAGCATCAGAAAAGTTGGTAACACAGTCAAAAGAACAGCCGAG  
AGTCCGTGTGTTGAGTATAATCTCGAATTGGGAAATATCCAGACGATTTGGAA  
TCGAACAATCAAAGTCTTTATAATAGATTGACAAAAATATTGGAAGCTGGAA  
ATTGGAGAATTGAAAACCTCGAAGAAAGTGTGTGGCACAATGTCCGACAGTCCAATG  
GCAACAATTTCTTGAAAAGTGATGAAATGACAAATAAAATTCGAGCGAACTTT  
ATCTGCAGAACTCGGAGAGACGATCAATGAGAATATTCTAAATATCACTATAA  
TGTTGGAATAAAGAAAGTACGAGAGGAGGACTTGTGAAATGTGATTCTGCG  
GGAAGAATTTCTGATTACAGTTTCTGTTTCTGTGTTGAACTGATCCTTGTTCCTA  
ATGGATATGATCGTCCATTTCATGGAGTGCTGAACTGTGTGAGTACATCCATT  
ATGTTTCATAAAGTAAATGATTTGATGATAACACATGAGAAACAGCTAAGTCGT  
CAAAATGGTTCAACATTTTAGTCGAATTGAAGAACTGTAATTGAGTATTTTCG  
TGGAAGTCTGATAGTCCATTATGCCCAATGGTTGTGAGGTGTCCATTGCACATT  
TCAAGAATTTCGAGAGGATTGGCTGATAAATTAATCTCGTACTCACCATAAA  
ATTCCTCCAATCAAGAAGCTGATGATCTACGAGACGAACTTGGAAAGACCTATA  
GTTCTCTCAAAATCAAACTTCAAGAACTCTAAGAAATTTTTTGAAAAGAACTTAT

FIG. 5 PAGE 1 OF 2

TCACCCGCCGCTCGTCCGACTTCAAGATCTCACTGATCGTGTTCATCGGAGCTCGTG  
CAAAATCACAAATGCTGGTCACTTTTCGATTATCTTCTTCGCAATGACACTTTGATT  
TTTATGGATAGACATCTTGATCAAATTCTTCTTGTTCGGTGTTCATTATGAA  
GATAAATGAGTCATCAATGCTTTTCACGGAAATAATGGCTCAATATCGACGACA  
ATCAGCCAAATCTTCTGGTCTACCGAAGTGTTACAGTATCCAGAACAACCTG  
AATCCCGAAATCCACAGGCAGTAAACACGAAGGAGACAATTTTGAACGCTT  
GAAGGTCCACAAAAAGAAAAACGACAGTTGATATAATCAAATATTATAATA  
TCGAGTTTCGGGATCGTATCAAGTATATTATCGGTCAAATTGATAGTCTTCAGA  
TGAAGATTGATGGAATCGCGGTTCGAAACAGAACTCGGATTGATCCCTGTTCGA  
GTTTATTTAACACATAAATTATCGATTCAAACGCTTCCAAAAACGAAACACGGA  
GAGTCGAAACAAGAAAGAGCTATTCCGAACCTTGAAAAATCTGGAATTACGATC  
GCTATGGAACGGTCTGGAGATTAAAAATGATTGTTGGAATACTTTGAACTTTTT  
AATGCATTTTIGATTAAATCATTTAGTACTTCTTTCTCGTCTATTTTTTATCTTT  
TCCTTCAAATTCAGGCAAGTAATTATACTTTCCATTCTAATTGATTGCTTCAA  
ATAGACGTCTAGTTATATTCAAAACAATCCCCCTTTGAATTGGAATCTTCAAAT  
ATCGTATTAAATATTAATTGTAATCATTTTTTACAATCCCCCATGCCATTATT  
GTTACTGATTTTTTCTCTTTTAAACCATCATCGATAAATTCATTTTACAGTTAT  
AAAAAAAAAAAAAAAA

SEQ ID NO:4

FIG. 5 PAGE 2 OF 2

SEQ ID NO:5

FIG. 5

&gt;lin-53 protein

MATLEDGTSSEDRVANDENKWKNTFFLYDLVMTALEWPSLSVQWLPDVAKDN  
 SDHTERLLLGTHTSDEQNELLISKCNPTDDAQFDASRYDTERSEYGGFGAVNGKVE  
 PDIRDHEGEVNRARYNPFQKSNHATKSPHADVYEDYLKHS AVPRDNTFNPLRLK  
 GHTREGYGLSWNPKEGLELSASDDQTVCHWDNANQNVAGELQAKDNTFGHESV  
 VEDVAWHVLEHDGVFGSVGDDNKLLWDVVRTSTPCHCDASHSAEVNCLAFNPYSEFI  
 LATGSADKTVALWDLRLNLRMKLHSHFESHDEIFQVQWSPHNETILASSGTDKRLHV  
 WDLKNGEDQSAEDAEEDGFFELLFHHGGHTAKISDFSWNPNEPWVVCVSEDNLLQV  
 WQMA DNTNENVEETPADVVERQQ

SEQ ID NO:5

FIG. 6

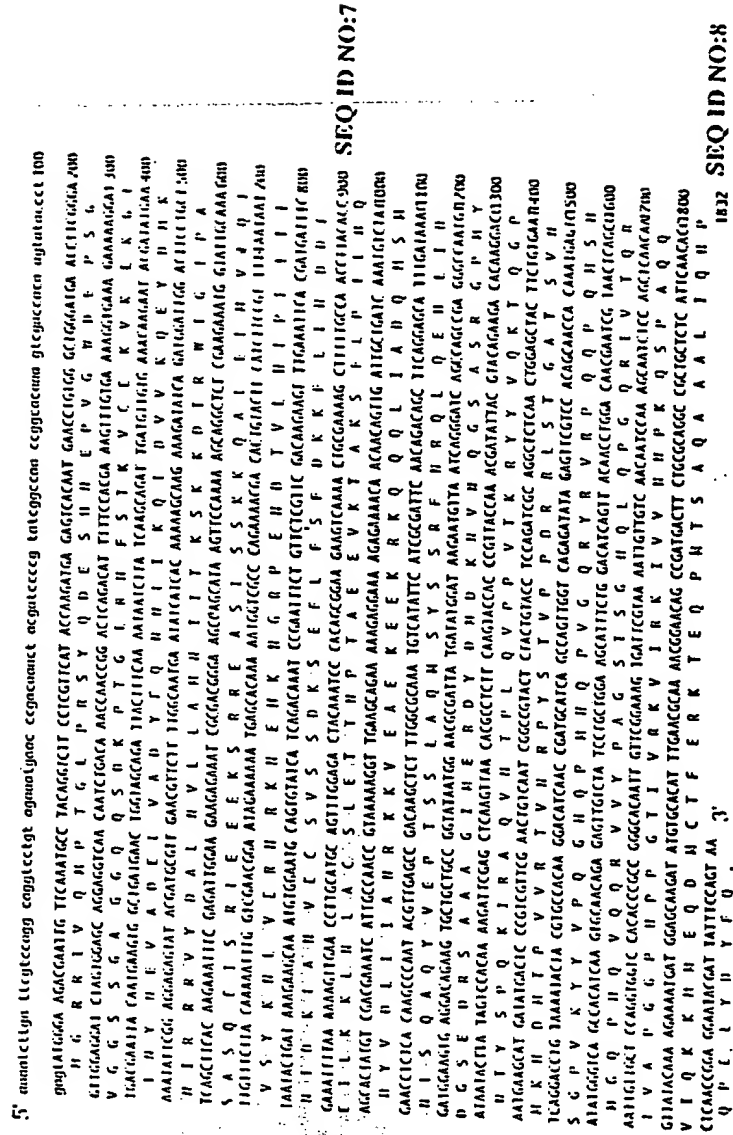
&gt;lin-53 cDNA

GAAATGGCCACTCTTGAAGATGGAACCTCCGAAGATCGCGTCGCAATGATGAAT  
 ACAAAATTGGAAAAAGAACACCCCGTTCTTGTACGATCTCGTAATGACACATGC  
 GCTTGAGTGGCCCTTCACTCAGTGTTCATGGCTCCCGACGTTGCGAACGATAACAG  
 CGACCATACTATTTCATCGGCTCATTCTTGGAACTCATACTTCAGATGAGCAGAATC  
 ACTTGCTTATTTCTAAGATCTGTATGCCAACGGATGATGCCAATTGATGCATCT  
 CGTACGATACCGAGCCGCAGTGAATACCGTGGTTTCGGAGCTGTTAACGGAAAAAGT  
 GGAACCCGATATTCCGATTAAACCACGAAGGGGAGGTTAACAGAGCTCGTTACATG  
 CCTCAAAAGTCGAATATCATTGCTACAAAGTCTCCACATGCTGATGTTACATTT  
 TCGACTATTTAAAGCACTCTGCTGTTCTCTCGTGATAACACGTTCAATCCGCTTATCA  
 GACTGAAAGGACACACGAAGGAAGGCTATGGATTATCATGGAATCCAAACAAA  
 GAAGGTTTGATTCTATCAGCGTCAGATGATCAGACAGTTTGTCAATGGGATATCA  
 ACGCAAATCAGAATGTTGCCGGGAATTGCAACCGAAGGATGTTTTCAAAGGTCA  
 CGAGTCAGTCGTTGAAGATGTTGCTTGGCACGTTTTGTCATGATGGTGTCTTCGGATC  
 GGTGGTGACGATAAGAAATTGCTCATTGGGATGTGCGCACAAAGCACTCTGGAC  
 ACTGTATCGATGCTCATTCTGCCGAAGTTAACTGTCTCGCATTCAATCCATATTCCG  
 AATTCTATTCTGGCCACCGGATCAGCTGATAAAACTGTCGCTCTTTGGGATCTACGT  
 AATCTACGAATGAAACTTCACTCATTGAATCAGATCGTGATGAAATCTTCCAAG  
 TTCAGTGGAGTCCACACAACGAGACTATTCTTGCATCCAGCGGTACTGATAAACGT  
 CTTCATGTGTGGGACCTATCTAAGATTGGAGAAGACCAATCTGCCGAAGACGCGGA  
 AGATGGTCCACCAGAGCTGTTGTTTATTACGGTGGGCACACCGCCAAGATCAGCG  
 ATTTCTCGTGGAAACCCGAACGAGCCTTGGGTTGTGTGACGTGTGTGAGAAGACAAT  
 ATTTCTCCAAGTGTGCGCAAATGGCTGATAACATATACAACGAAGTTGACGAAGAA  
 ACTCCAGCCGATGTGGTAGAGAGACAACAGTAAAAATACGTGAAACGCGCTTAAA  
 TTATTGTATTAACTTCTATCCTTCTTTAATTTTGCATCTCAACAAATTGTTTAT  
 CTTACCAATTTATTCAAACGCATATTCTTCACCAACTAAGTTTTTAAAGTTAAAA  
 TGTTACCTTGAGATATGATCATATTTTGTGAACTGAAATAAATTCGATGACCA  
 TTGTCAAAAAAAAAAAAAAAAAAAAA

SEQ ID NO:6

FIG. 7

PARTIAL *lin-55* TRANSCRIPT WITH PREDICTED TRANSLATION



Notes: The 3' untranslated region has not been sequenced.

FIG. 8

*C.elegans* E2F-1 Protein Sequence

1  
MELQKALEMTKQSSIKNNLMLGLDNELDFDFDFDEDEDLDQPQMGR  
ADKSLGLAKRFIRMIQYSPYGRCDLNTAAEALNVRQRRYDITNYLE  
GIGLIEKREKNNMIQWKGGDFMLNVKEGKRLSATTEEDRMIEQLKAEIE  
QLNKEEELIEQRQRWLQCSLRNMITESVENNKLSYVLRSQLAEIQGSDLT  
IGIQRVGTQVRLSDPEQVEHGGPSWCYLKDPGGLRAAIVSNHELHDF  
VQRERAKRPGEEHVDADAPDENIDDSRYRNRRITINDDEMFGFEQKVP  
AMKHLEPPASDDYVYSTGDEYRGDSIVDLYGD  
324 SEQ ID NO:9

FIG. 9

FIG. 10

*C.elegans* E2F-1 cDNA Sequence

1  
GTTTGAGCCATGGAAGACTCGTACAACGACATGGAAGACCCCGGC  
TCCGCCAATTATCTGACATGGAGCTTCAAAAAGCGCTGGAATGA  
CCAAACAGAGCTCGATAAAGAAACAATTTGATGCTCGGGCTCGACA  
ATGAGCTTGACTTTGATTTTGATTTGACGAGGATGAGGACCTGGAT  
CAACCACAAATGGGCACACGAGCCGATAAATCGTTGGGATTGTTG  
CGGAAACGATTTATTCGAATGATTCACTACTCACCGTATGGAAGAT  
GCGATTGAACTGCGCGCCGAGGCGCTCAATGTCCGGCAAAAGC  
GACGAATCTACGATATTACGAATGTTCTCGAAGGAATTGGTCTTATT  
GAGAAAAGAAAGCAAGAATATGATACAGTGGAAAAGCGGTGATTTT  
ATGCTAAACGTGAAGGAACGGGAAACGACTATCGGCCACAACAGA  
AGAAGAAGATCGAATGGAACAATTAAGCTGAAATTGAGCAATT  
AAATAAGGAAGAAGAGCTCATTGAGCAACGTCAAAGATGGCTTCA  
GCAGAGCTCCGAAACATGACAGAATCCGTGGAGAACAACAAGCT  
CAGCTATGTGCTCCGTTCACAGCTCGCCGAGATTCAAGGCTCAGAT  
CTTACGATTGGAATTCAAACGAGAGTCCGGCACACAAGTTCGGCTCA  
GTGATCCGGAGCAAGTCGAGATACACGGTGGACCATCTTGGTGTTA  
CCTGAAAGATCCCTCTGGACCCCTCCGAGCCGCCATCGTTTCCAAC  
CATGAGCTACATGATTTTGTACAGAGAGAACGAGCAAAACGGCCT  
GGTGAAGAGCACGTTGACGCTGATGCTCCAGATGAAATGATGGAT  
GATTCAAGATATCGGAATCGGCGGACGATCAATGATGATGAAATG  
TTTGGTTTGTAGCAGAAAGTCCCAGCGATGAAGCATCTGGAGCCAC  
CACCGGCCAGCGATGACTACGTTTATTCGAGCACCGGAGACGAGT  
ATCGAGGAGATTCTATAGTCGATTGTACGGAGATTAAATTATTTAA  
TATTTTATTTTAAATTTTGAATTTCTGGGACCAATTTCTCATTTGACATC  
TATTCATTTACTCCAAATTTCCAAATTTTCCCCAAAAAATTATCGA  
TGTTTCGGCTCCAAATGTTATTATTTTCCCATCCACAGTCCCCACAC  
AATTCATAATGTGCCCTCTGGAGAAAACCTAACGTATTTCAATTTCTA  
TCCCAATTTTATTTTCAAAAATTTCTCAGATTTTAAATTTATTG  
TCACACTTTTTCTGTATTCAACTGAATTTTCACTTGGATTGTGA  
CGTTTTTTTTTGTTCATTTTAATGGATTTTCACTTGAACCCCA  
ATAAAAACGGGATAAATCGACGTTTTTGAATAAAAAAAAAAAAAA  
AA

142S

SEQ ID NO:10

FIG. 10

## LIN-52 Protein Sequence

1  
MSRPLGFIGYFQDDENFVQQMIKKSNAEQAKMLEQQKKMLECTET  
MPEESEPVNPKCLDFEEAFQSESVSKGYESPYNISFLKEDAVTVNTMSH  
CPADDIAKLIRNIQNSVYTLGIEEARQCRRGKLLNVLKPTGSASPRYLQP  
TPPKNVAEETIGSQ  
161 SEQ ID NO:11

FIG. 11

*lin-52* cDNA Sequence

1  
ATGTCGGCTCCGCTAGGATTTATTGGATACGAATTGGAGATGACG  
AAATGTTTCGTCCAACAATGATCGAAAAGAAATCAAACGCAGAAC  
AGGCGAAAATGCTTGAAACAACAGAAAAAGATGCTCGAATGCACCG  
AAACAATGCCAGAAAGAAAGTGAGCCAGTTCCAATGAAATGCTCTG  
ATTTTGAAGAAGCATTTCAAACCGAATCAGTATCAAAAAGGTTACGA  
ATCGCCATACAAGAATATTTCTGTTTCTCAAGGAAGATGCTGTGACT  
GTTAATACAATGAGCCACTGCCAGCCGACGATATCGCCAAGCTCA  
TCCGAAACATTCAAACTCGGTGTACACTCTTGGAATCGAAGAAGC  
TCGCCAGTGCCGACGTGGAAAGTTGCTCAACGTGCTGAAACCCACT  
GGCTCGGCTTCTCCGAGATATTTCAGCCAACACCACCGAAAAATG  
TAGCGGAAGAAACGACAGGAAGCCAGTGAAATTGAA  
493 SEQ ID NO:12

FIG. 12

*lin-54* Protein Sequence

1  
MNQGEIVYQDDDDYYT ESEYDNYEEGAEFIEVNGQLVPHNPNLQAAQ  
QNRPGTSSMIQQHNRSMEVNQGLVKDEPIDTSSHRVYVPPRPVQRKL  
WKLFPQGPSTPGSSQYTVRNLSNLGSPSMYDRQPASLPRTVOPMGLM  
GNSEQRKVYIDMKDHVSHIRLKTKKVFAFGORKPCNCTKSQCLKLYC  
DCFANGEFCRDONCKDCFNNEFYDSORSKAIROSLERNPNAFCKIGIA  
RGGITDIERLQKGGCHCKKSGCLKNYCECYEAKVPCTDRCKCKGQNT  
ETYRMTRYKNSGGAVSNTNALMSLTNASSTATPDSGPGSVVTDHGGDD  
YEDMLLSHKPKVEMDFRFPWYMTDEVVEAATMCMVAQAEALNY  
EKVQTEDEKLNMEKLVLRFGRCLEQMTNTTTELTDLDAAPTDDIPG  
PSTST5  
458  
SEQ ID NO:13

FIG. 13

*lin-54* cDNA Sequence

1  
ATTTTCAGTGTGACAATCAATCAACCAGAAATCGTTTATCAAGAC  
GACGATGATTATTACGACGAATCGGAGATATACGATAATTATGAAG  
AAGGTGCCGAATTTATCGAAGTTAATGGACAGCTTGTGCCTCATAA  
TCCAAACTTACAGGCGCAGCAAAATCGTCCGGGAACCTCGAGTAT  
GATTCAACAGCATAATCGATCAATGGAAGTTAATCAGGGATTGGTC  
AAAGACGAACCAATTGATACATCATCGCATCGCGTCTACGTCCCCC  
CTCCGAGACCAGTTCAGCGAAAACCTTTGGAAGCTTTTTCAGCCTGG  
GCCGAGCACTCCCGGATCGTCTCAGTACACTGTGCGGAATTTGTCC  
AATTATCGGGTTCACCTTCAATGTACGATCGACAGCCCGTTCATT  
ACCTAGAACAGTGCAACCAATGGGCTTGGAGATGGGAAATTCGA  
ACAGCGAAAAGTTTACATCGATATGAAAGATCAGTTAGTCATATT  
AGATTGAAAACATAAAAAAAGTATTTGCACCTGGCCAGCGGAAA  
CCATGCAATTGACGAAATCTCAATGCCTCAAGCTCTACTGTGATT  
GTTTCGCCAATGGAGAGTTCTGTCTGACTGCAATTGCAAGGATGT  
CACAATAATATAGAAATACGACAGTCAGCGTTCAAAAGCCATCCGT  
CAGTCACTTGAGCGAAATCCGAACGCTTTCAGCCAAAAATTGGTA  
TTGCTCGTGGAGGTATTACCGACATCGAACGCTTTCATCAGAAAGG  
ATGTCACGTGAAAAAGAGTGGTGTCTGAAAAACTATTGTGAGTGT  
TATGAAGCAAAGGTCCCGTGTACCGATCGATGCAAGTGCAAAAGGA  
TGTGAGAATACTGAAACATACAGAATGACAAGATACAAGAATCC  
GGTGGTGCCGTGTCCAATACGAATGCCCTGATGTCATTACCAACC  
CTTCCAGCACAGCGACTCCAGATTCTGGTCCGGGAAGTGTGGTGAC  
CGATGAGCATGGAGACGACTACGAGGATATGCTTCTTTCCGATAAA  
CCGAAGGTCCGAGATGGATCTAGACCTTCCCGTGTACTATATGA  
CCGATGAAGTCGTGAGGCAGCCACTATGTGCATGGTGTCTCAAGC  
TGAACAACTTTAAACTACGAAAAAGTGCAAAACCGAAGACGAAA  
AACTCATCAATATGGAGAAGCTTGTCTTCTGTAATTCCGCCGCTGT  
CTCGAACAATGATCACAACACAACCTGAGCTCACACAACATCTT  
GATGCCGCTCCAACCGATGACATCCAGGACCATCTACTAGTACTT  
CTTAACATTTCCGATTAAATTAATTAATCAATTTTATCACAGTTGCGC  
GATCTTTTATCATCTCACCCTCACACAATCTTTCCTTCTCCCCCTC  
CTCTCAATGCTTTACAGATTACAAGTTGCTTCTTTCAAAGTTGTG  
AAATAAAAAATGATCAGAAAAATTTGTTTCAT  
1503  
SEQ ID NO:14

FIG. 14



*lin-52*

*lin-52* maps on LGIII between *unc-16* and *unc-69*.

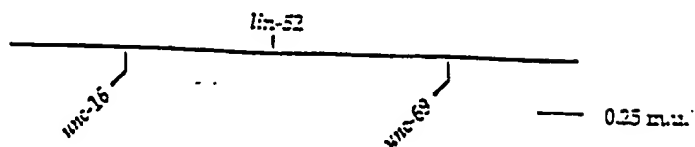


FIG. 15

*lin-55*

*lin-55* maps on LGII between *rol-6* and *unc-4*.

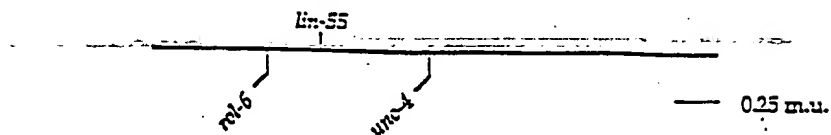


FIG. 16

FIG. 17

FIG. 17 shows a partial LGIII map.

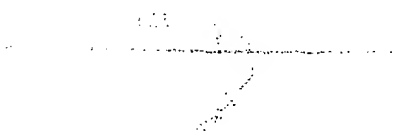


FIG. 18

*lin-37* message is present in both  
embryonic and mixed-staged RNAs

Embryonic  
Mixed-staged

1 kb →

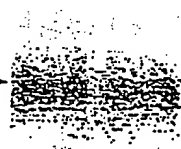
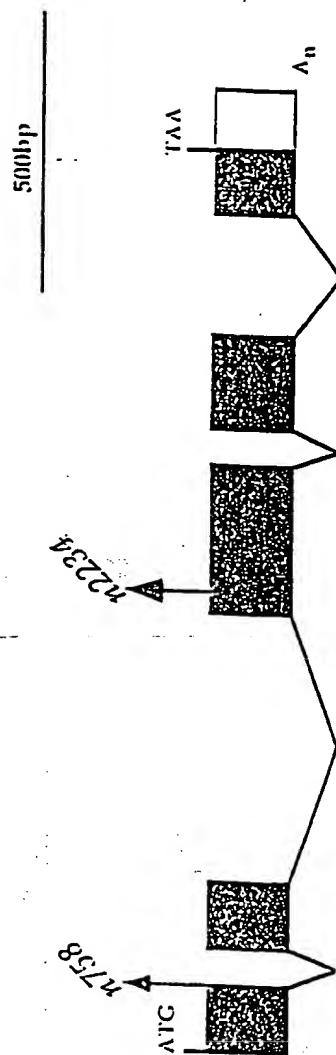


FIG. 17

*lin-37* Gene Structure and Mutations



n758 G → A, splice donor

n2234 AAG → TAG, K105amber

FIG. 18

*lin-37::GFP*

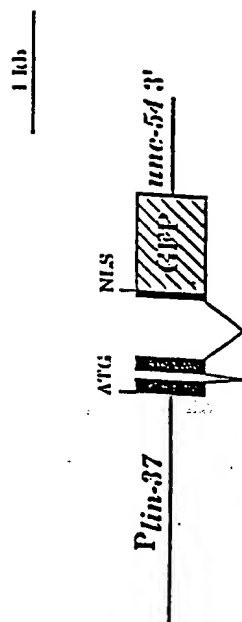


FIG. 19

# lin-37 Rescue

The *lin-37* minimal rescuing fragment contains five ORFs as predicted by GeneFinder. Only the ORF C.cand2 is required for rescuing activity, therefore is likely to be *lin-37*.

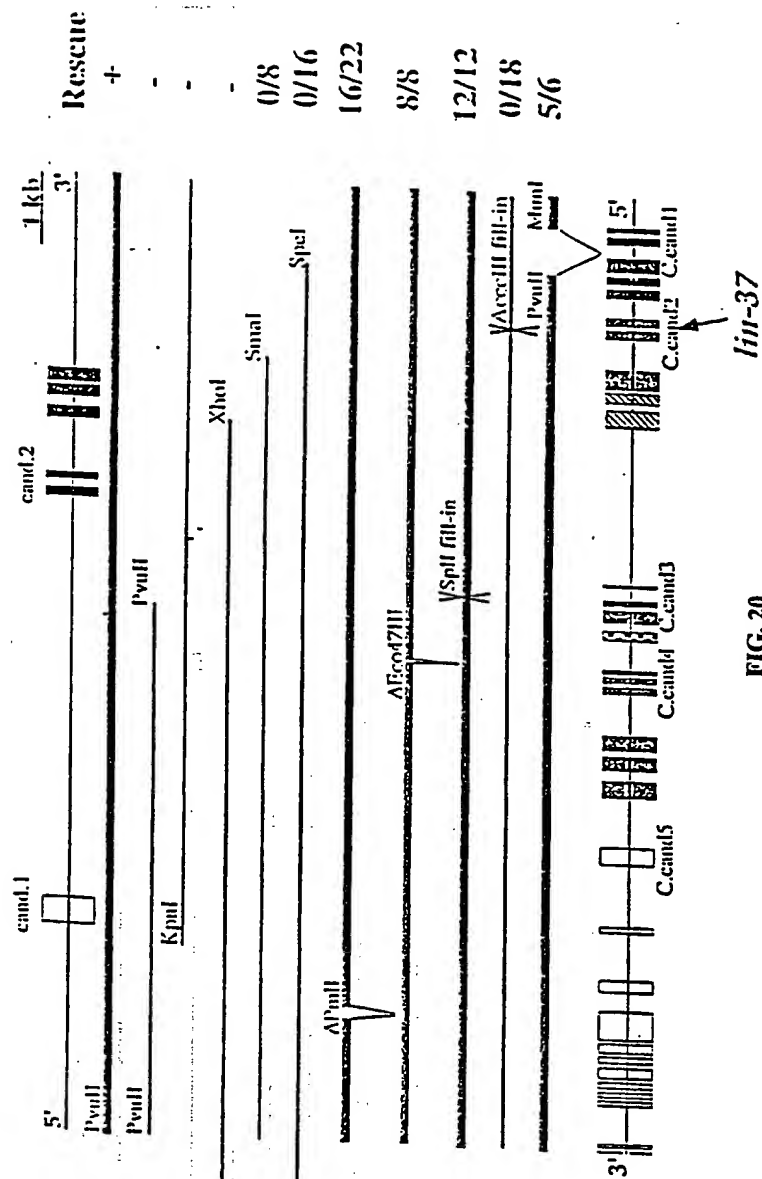


FIG. 20

*lin-37* encodes a novel 32 kD hydrophilic protein

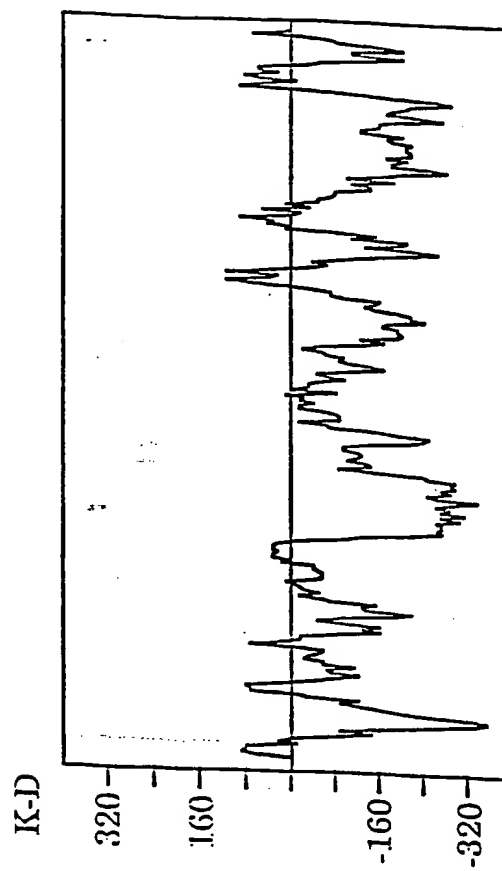


FIG. 21



Results indicate that LIN-36 is able to self-associate. No other interactions among the synMuv's were detected.

		PREYS			
		<u>lin-9</u>	<u>lin-15A</u>	<u>lin-15B</u>	<u>lin-36</u>
BAITS	SNF1 (- control)	-	-	-	-
	lin-9	-	-	-	-
	lin-15A	-	-	-	-
	lin-15B	-	-	-	-
	lin-36	-	-	-	++

FIG. 23

## Characterization of Molecular Interactions of the synMuv Gene Products

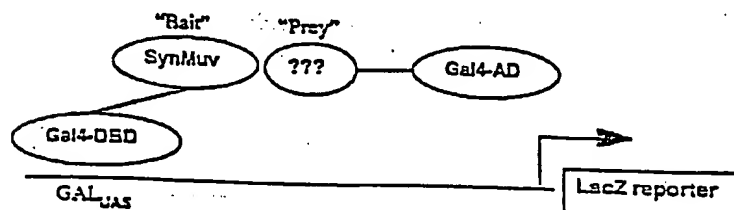


FIG. 24



## Future Search for Additional synMuv Alleles

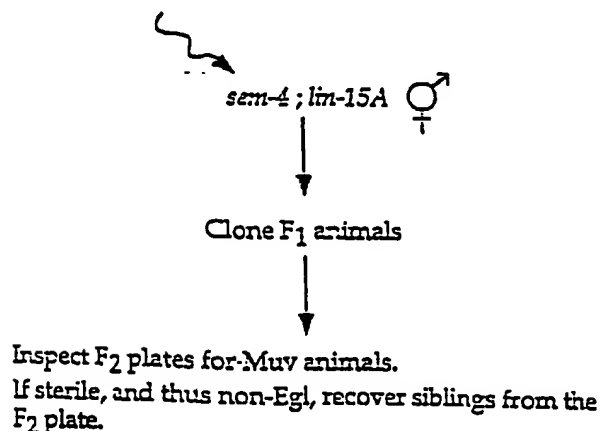


FIG. 25

## Sequence of *Mus musculus* cDNA clone vp19d01

1  
 GTGCTAAAACAATTGGTGAACCAGACTTGCCCAAAGTTGTTAATAT  
 TAACTTCTGTCCATCTGGTTTAAGGGTCTGATTGCCAAGTTAAGAT  
 CAGATGTCTGTGATACTTGTGTTAAAATAATCTGATTGGCTGATATA  
 GTCACAGGAGTCTGAGCACCAAGTTTTGAAGGCCACTTGGAAATG  
 CTGGTTTCACTGTGGTATTAGAATCTGCTTTAGAAACTGTGGTATTC  
 ACTGCAACTTGGTTAGTGTGGTACTGTACACTGTGATTGGTTCCGT  
 GGAAATGGGCGTGGCTGTAGAGTCACCGGTAGAATTTATGTTGACA  
 ATTCTTCCAGCTCTGTCTCCATGGGAATTGGGGATGACACAATTAC  
 AGCCTCAATACTATCCTCATCCACTAACGTTATAGCAGTGTCCATTA  
 TGTCGTCTGGAAGCAAACCTATTCACCTCGG

449

SEQ ID NO:15

FIG. 26

*Homo sapiens lin-54-like cDNA Sequence*

1  
AAACTGGTCCAAGCTGAATTCCGGGGAAAAGGTGAAAATGGCGGA  
TCTTTCGAAATACAATCCCCGGCCCTGACATACCAGAGCGCGCGG  
GACGGCGACGTGCGCAACCCGGCTCCCTTCTCGGTCCCCGGGTACC  
CTGGACCGCTCCAGTTTGGACAACTGGGAAAAGGAAGCTGCTGGC  
CAGAGCTATCGTGCACGGGCAAAACAAGCCCCCGCGGGCTCGGG  
ATTGCCCAGGACCTTCCGAGCCCTACCTCGGAGCCCGAGGGAG  
GAGAAAGCGGCAGCCGCTGGAGTGCCTGATCAACTTTCTAGCGGG  
AGAACGATCATGGAGGTGGTCCAGCTGAGGTGAATAGTTTGCCTC  
CAGAGGAAATAATGGACACTGGTATAACTTTAGTGGATGATGATAG  
TATTGAGGCTGTTATTGTTTCATCCCCAATTCCCATGGAGACAGAAC  
TGGAAGAAATTGTCAACATAAATTCTACTGGTGACTCTACAGCCAC  
GCCCCATTTCCACGGAACCAATCACAGTGTACAGTAACCACTAAC  
CAAGTTGCAGTGAATACCACAACTACTAAAGCAGATTCTAATACCA  
CAGTGAAACCAAGCTTTTCCAAGTGGCCTTCAAAAACCTTGGTGCTCA  
GACTCCTGTGACTATATCAGCCAATCAGATTATTTTAAACAAAGTA  
TCACAGACATCTGATCTTAACTTGGCAATCAGACCCTTAAACCAG  
ATGGACAGAAAGTTAATTTTAACTTGGGCAAGTCTGGTTCACC  
AATTGTTTTAGCACTACCCCATAGCCAACCTACCCAGGCTCAGAAA  
GTACAACTCAGGCCCAGTCAGGAGATGCTAAGTTACCACCGCAGC  
AAATTAAGTAGTTACCATTTGGAGGAGGCCAGAGGTGAAAACCTG  
TCATTGGTGTCTCAGCATTGACCCAGGAAGTCAACTGATTAATAC  
TACAACTCAGCCCTCTGTGTTACAGACCCAACAGTTAAAAACAGTA  
CAGATTGCTAAGAAGCCTCGAAGCCCAACCTCTGGTCCAGTAATCA  
CGAAGCTGATCTTTGCAAAACCAATTAATAGTAAAGCAGTTACAGG  
ACAGACAACCTCAAGTTTCAACCAAGTCATTGCAGGTAGGGTCTT  
TCACAGTCTACTCCCGAACTCCATCAAAGACCATAACAATATCTG  
AAAGTGGTGTATTGGATCAACTTTAAATCTACAACACAGACACC  
AAATAAAATAGCCATCTCACCTTTGAAATCGCCAAATAAGGCAGT  
GAAATCAACTGTGCAGACCATCACTGTTGGAGGAGTGAGCACATC  
ACAGTTTAAGACAATTATCTCTGCGCAACTGCTCCCAATGTCCAG  
CAGATTCAAGTGCCTGGAAAGCAAGTTTCATTATGTCGACTTGTAC  
TGCCACATCAGCCAGTAGCTCAACCCAGCCAGTTAGTCAGAATCCC  
AGTACAAACACTCAGCCTTTTACGCAAGCAAAGCCAGTGGTTGTTA  
ATACAAACCCAGTGGCGATGTCAGTTCCAATTGTCTCAGCTCAGGC  
TGTCAAACAAGTTGTTCAAAACCAATCAATCCAACCTTCAAAATA  
GTAACCTACTAGCCAGCCACAGCAACGGCTTATCATGCCTGCCACAC  
CACTGCCACAGATCCAGCCCAACCTCACTAACCTGCCACCAGGCA  
CTGTCTTGGCACCAGCTCCGGGAACAGCGAATGTGGGTTATGCAGT  
GCTTCCAGCTCAGTATGTTACTCAGCTACAGCAGTCTTCATATGTAT  
CAATAGCAAGCAACTCTACCTTTACTGGAACATCTGGTATCCAGAC  
CCAGGCACGGCTTCCATTCAATGGCATAATCCCATCAGAGTCCGGC  
AGTCCGCCCCGAAAGCCCTGTAAATGTACAAAATCACTGTGTTGA  
AATGTATTGTGATTGCTTTGCAAAATGGTGAATTTTGAACAACCTGC  
AATGTACTAATGTGTACAACAATTG

2000

FIG. 27 PAGE 1 OF 2

*Homo sapiens lin-54-like cDNA Sequence, cont.*

2001

GAACATGAAAATGAAAAGGCAAAAAGCAATAAAGGCATGCCTTGA  
CAGAAATCCAGAAGCCTTTAAGCCTAAGATAGGGAAAGGAAAGG  
AGGGAGAATCTGATCGACGTCATAGCAAGGGTGTAATTGCAAAAC  
GATCAGGATGCTTAAAAAATCTACTGTGAATGCTATGAGCCAAAAAT  
AATGTGTTCCTCAATATGCAATGTATTGGCTGTAAGAATTTTGAAG  
AAAGCCCGGAAAGGAAGACATTGATGCCATTGGCAGATGCAGCTG  
AAGTAAGGGTACAGCAACAAACAGCAGCCAAGACGAAGTTATCCT  
CTCAAATTCAGACTTGCTTACTAGGCCAACACCAGCTTTAAATAGT  
GGAGGCGGAAATTTGCCATTACATTTGTAATAAGGAANTANTCTG  
AAGCCACNTGTAATTGCCNCTTGCCAGGCAGAGCAGGCAGACA  
AGAAGGGAAATCAAAAGGCAGCAGCGGAACGGATGATACCTTGAG  
GAATNCGGACNATGTTTATGANTGTTCATCAACTCTGCAGGAAAG  
GCAAAAANTGACCCCTGTGCCCCATGAATTGCTAACTCTTGACAAA  
AGACTGATAAATGGAACTGTACAGAAAATTTAAGGTGCAGGGACA  
CTTGATTTTCTGGAAGAAAAACAACTACTGTATTTTAATTCAGTCT  
TGTTTTAAAGACCTGAAATTATAACTGAAGGAGAAGAAATTTT  
AAATGAGGAAATTAGTACATTTTAAATCTTAGTTAAATCTGCTTATG  
CCCCTTCTAAATTGAATTTTTCTNTATTATATAGATTTTTTAATTTGC  
TTGGGTTCCTTAAGAATTAGATGTCTCTTCTGATACCTTTGACAAA  
AAATGTTTATAAATTCATATAATTTATAATGTATGGTGTGTATGAC  
TTTGTTAATAGAAAAGCCAAAGCAGCAGTGGTTAGCACCCATTCTT  
TGGGACTTGATCTAGAATATCTGCAGACAGAATGTTACATAAACAA  
ATTCTTATGAAACACATTCAAATGACATTTTGTATTTAGAAAAGGA  
CTATCTTTTAAAGAAAAGCAGCCTTTTAGGGCCGATTCTGGAATA  
ATATCTGTGTCACTTTGGGAATGTCAGAAGGGGAAACAATCCCC  
AGGCACACTAAAAATTTTTAAAGTTATTTAAAAAAAACATATAAAA  
TATTAAAGGACAGTAAATCTCAGAGGATGGCCAATGTGTTTCTATA  
ATAAGGAAGGCTAACAGATGCTCTGGGCTGTCTCCATTTTCTTTCA  
AAGAGGTGGTATGTATTTGAAGTAATAAATTGTCAAAGTGATTACT  
GGGTACTATTAATAATGATAGGTGGATATAAATGGAAAGTAAACATTA  
TGTAGTGATAATATAGAACCTCACATAGTAATCAAGTATAAAATTT  
GGCATGGGTGGAGAAACAAGNATCGGGAAGCTGCCAAAGATGA  
ATTTAGAGAAGTTTTCTATACAATCAATTATTTTACAGACTTTT  
TTCCCGGAATTCITTTGCT

3535 SEQ ID NO:16

FIG. 27 PAGE 2 OF 2

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/11043

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12N 5/00; C07H 21/04

US CL :536/23.1; 435/320.1, 325

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.1; 435/320.1, 325

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, BIOSIS, EMBASE, MEDLINE, DERWENT BIOTECHNOLOGY, CAS

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HEDGECOCK et al. The ncl-1 Gene and Genetic Mosaics of <i>Caenorhabditis elegans</i> . Genetics. November 1995, Vol. 141, pages 989-1006, see the entire document.	1-6, 11-18, 25

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

Special categories of cited documents:	
*A* document defining the general state of the art which is not considered to be of particular relevance	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*E* earlier document published on or after the international filing date	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	*A* document member of the same patent family

Date of the actual completion of the international search

05 OCTOBER 1998

Date of mailing of the international search report

23 OCT 1998

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

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Authorized officer

BRUCE CAMPBELL

Telephone No. (703) 308-0196

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/11043

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☒ Claims Nos.: 7-10  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:  
  
no sequence disk was submitted.
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6A(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
  
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
  
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
1-6, 11-18 and 25

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/11043

## BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-6, 11-18 and 25, drawn to (A) a nucleic acid encoding a SynMuv polypeptide selected from the group consisting of LIN-37, LIN-35, LIN-55, LIN-53, LIN-52, LIN-54, and E2F-1; (B) vectors containing the gene and cells containing the vector as well as cells containing the nucleic acid as found in a patient; (C) the first method of obtaining the nucleic acid polymers (A) above; and, (D) a first method of use of the cells in (B) to identify compounds that modulate cell proliferation.

Group II, claims 19-21, drawn to any SynMuv polypeptide (claims 10 and 21 and composition where the first listed species is LIN-54 in claim 20.

Group III, claims 22-24, drawn to a method of modulating cell proliferation by administration of any one SynMuv protein.

Group IV, claim 26, drawn to an antibody that binds to any one SynMuv protein.

Group V, claims 28-33, drawn to a method of diagnosis of a cell proliferation disease via determination of the presence or absence of genetic mutations and/or gene expression and is a second process of use of the polynucleotides, vectors and cells containing genetic material encoding any one SynMuv protein.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be searched, the appropriate additional search fees must be paid.

The species are as follows: (1) LIN-37, (2) LIN-35, (3) LIN-55, (4) LIN-53, (5) LIN-52, (6) LIN-54, and (7) E2F-1.

The claims are deemed to correspond to the species listed above in the following manner:

Claims 1, 4-6 and 11-18, all in Group I refer to one of LIN-37, LIN-35, LIN-55, LIN-53, LIN-52, LIN-54, or E2F-1.

Claims of Group I which are claims 2 and 20 of Group II refer to LIN-54.

Claim 3 refers to LIN-37, LIN-52, or LIN-54.

The following claims are generic: 4, 19, and 22-33.

The inventions listed as Groups I through V do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons.

The claims of Group I are directed to polynucleotides, vectors containing the polynucleotides and cells containing the vectors. These claims do not indicate nor require for their practice, a protein nor any antibody, nor methods of diagnosis.

The Group I claims refer to a "substantially pure nucleic acid encoding a SynMuv polypeptide". The application written description at pages 11-12 defined "substantially pure DNA", i.e., "substantially pure nucleic acid", as being:

"free of the genes which, in the normally-occurring genome of the organism from which the

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/11043

DNA of the invention is derived, flank the gene. The term therefore includes ... a cDNA or genomic or cDNA fragment produced by PCR or restriction endonuclease digestion)..." and therefore a gene library anticipates at least claim 1. Anonymous. C. Elegans and C. briggsae arrayed genomic DNA libraries. Worm Breeder's Gazette. 01 October 1996. Vol. 14. No. 4, page 14. [Retrieved on 1998-07-31] Retrieved from the internet via <URL: <http://elegans.swmed.edu/wli/wbg14.4p14>>.

Thus, the special technical features do not define a contribution that each of the claimed inventions, considered as a whole, makes over the prior art.

As to Groups II and IV which are directed to polypeptides, a polypeptide and polynucleotide (Group I and V) do not share the same physical, chemical, or biological properties and function. Moreover, in Groups II and IV, the Group II proteins have different structure and function from an antibody. The antibody of Group IV is not used nor required for the practice of both Groups III and V. Groups III and V use different compositions and Group V is an alternative process of use of the polynucleotides in Group I.

The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: The genes *lin-37*, *lin-35*, *lin-53*, *lin-52*, *lin-54* and *e2f-1* that encode the proteins LIN-37, LIN-35, LIN-53, LIN-52, LIN-54, and E2F-1 are indicated in the application written description as part of a pathway (pages 2-3) and each gene does not have the same function nor polynucleotide sequence and encode different proteins.

REPORT